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***USSR: SPACE BIOLOGY &  
AEROSPACE MEDICINE***

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## USSR: SPACE BIOLOGY &amp; AEROSPACE MEDICINE

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## CONTENTS

Present Stage of Space Medicine .....	1
Results of Long-Term Electrocardiographic Monitoring of Cosmonauts ....	10
Improvement of Human Tolerance to Head-Pelvis (+Gz) Accelerations by Increasing Hydration .....	17
Effect of Spaceflight Factors on Biological Material Flown Aboard Cosmos-1514 Biosatellite .....	23
Investigation of Genetic Structures of Rat Germ Cells Following Flight Aboard Cosmos-1514 Biosatellite During Their Prenatal Development .	32
Morphological and Functional State of the Hypothalamo-hypophyseal Neurosecretory System of Rats Flown Aboard Cosmos-1667 .....	37
Investigation of Rat Skeletal Muscles Following Short-Term Spaceflight Aboard Cosmos-1667 Biosatellite .....	43
Morphological Study of Early Changes in Rat Bones in Simulated Weightlessness .....	50
Lipid Peroxidation in Rat Tissues With Exposure to Antiorthostatic Hypokinesia, Exercise and Immobilization Stress .....	55

Role of Carbon Dioxide in Correction of Coagulation Hemostasis Under Hypoxic Conditions .....	62
Effect of Cardioactive Compounds on Rat Myocardium Actomyosin Following Exposure to Accelerations .....	68
Morphological Manifestations of Primate Adaptation to Orthograde Statics and Walking Erect .....	73
Effect of Exogenous Cardiosynchronized Counterpulsation on Human Regional and Central Hemodynamics .....	79
Effect of Oxygen Inhalation on Respiratory Function During Exercise and Exposure to Added Resistance to Respiration .....	86
Polar Work Adaptability to Antarctic High Altitudes .....	92
Effect of External Electrostatic Field on Catecholamine Secretion by Rat Adrenals .....	99
Two-Dimensional Static Otolith Model .....	104
Combined Effect of Ionizing Radiation and Altered Gas Atmosphere on Central Nervous System .....	116
Effect of Hypercapnic-Hypoxic Test on Cardiorespiratory Parameters of Individuals With Neurocirculatory Dystonia .....	126
Oxidative Enzyme Activity in Healthy Subjects and Individuals With Neurocirculatory Dystonia During Graded Exercise .....	131
Hemodynamic Effects of Beta-Adrenergic Blocking With Obsidan in Clinostatic and Orthostatic Positions .....	136
New Book on Metabolic Aspects of Spaceflight Stress .....	144

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PRESENT STAGE OF SPACE MEDICINE

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (signed to press 23 Oct 87) pp 3-10

[Article by B. S. Alyakrinskiy]

[Text] Our country is celebrating the 70th anniversary of establishment of the world's first socialist nation in an atmosphere of dramatic increase in activity of the broad masses, under conditions of comprehensive restructuring of society.

The strategic course toward acceleration of socioeconomic development, qualitative transformation of all aspects of life of our society reflects the dialectics of development of social relations which open up a wide arena for initiative and creativity of the masses. This course, as stated by M. S. Gorbachev at the June 1987 Plenum of the CPSU Central Committee, is actually continuing the cause of the revolution through restructuring, through renewal of all areas of social life. And, of course, the success of restructuring depends on the extent to which its concepts are grasped by each member of our society, the clarity with which he comprehends its necessity and inevitability. Any undertaking, any progressive idea acquires reality only when it is grasped by the masses. All documents--instructions, directions, ukases--will remain only as pieces of paper if they are not grasped by the mind, the heart of the executor. Even N. V. Gogol wrote that "... an ukase, no matter how well thought out and how specific it is, is nothing more than a blank sheet of paper if it is not based on an equally pure desire to apply it to the cause in expressly the required way...."<sup>1</sup>

There is a diversity of forms, of concrete ways and means in the "perestroyka" [restructuring] process that is going on in our country, which has an integral content that is determined by the course of objective laws of social development, in this case, development of a classless, social society, the builders of which manifest the primordial desire of the human spirit for creativity, since, "... the more man becomes human, the less he will agree with anything other than infinite, ineradicable movement toward the new."<sup>2</sup>

The founders of scientific communism have repeatedly stressed that the substance of human creative forces is fully disclosed only in a society that is free of man's exploitation of man, in a society where there is harmony of social and personal interests. Restructuring, as it is taking place in our

country, is based on increasing the creative activity of the entire nation, strengthening conviction of the immovable nature of the lofty principles of socialism, the reality of implementing them and, as stated by M. S. Gorbachev at the June Plenum of the CPSU Central Committee, "expressly today, tomorrow, rather than at some remote future time...." There is a noble goal in the grand task of perestroika that is of paramount importance--to provide conditions for a full and happy life for the Soviet people, to preserve their moral and physical health. The struggle to reach this goal requires increased activity on the part of all members of society and, at the same time, implies strict, professional differentiation of efforts made. Thus, providing for the physical welfare of Soviet man is the moral and professional essence of the health care system, of Soviet medicine.

Much has already been done in this direction in our country. Major advances have been made in many branches of Soviet medicine. For example, the work of Soviet ophthalmologists (clinics of M. M. Krasnov, S. N. Fedorov), traumatologists, primarily the school of G. A. Ilizarov who wrote a new page in this branch of surgery, has gained broad recognition. There has been significant development of branches of medicine that were formed relatively recently. We should include among them, first of all, space biology and medicine, the inception and development of which were determined by the general development of cosmonautics. This proceeded at an exceptionally rapid pace in the Soviet Union, as evidenced by the priority of our country in most major human conquests on the road toward space exploration.

Great advances have been made in the Soviet Union in the field of space medicine: the duration of spaceflights is constantly increasing, and there are more frequent and longer episodes of extravehicular activity. All this is indicative of the increased complexity and intensity of work operations performed during flights and, consequently, of increased demands as to level of mental and physical work capacity.

The advances of Soviet researchers with regard to the problem of weightlessness are well-known and recognized. Spaceflights were, so to speak, a biomedical proving ground, which made it possible to conduct specialized research under conditions that cause manifestation of patterns of vital functions in both morphological and functional aspects. Each law of life will always be manifested in a more acute form under more rigorous conditions, it will be more prominent, more demonstrative. A complicated organism is more expressive in weightlessness than on the ground, it reveals the mechanisms of its functions, which permits effective intervention in the process of its adaptation to an altered environment.

Within a relatively short period of time, from the first manned spaceflight by Yu. A. Gagarin to the present, much fruitful work was done with respect to both investigation of the effect of weightlessness on biological systems (primarily man) and development of appropriate preventive measures. There is no need to analyze these developments. The duration of the most recent missions is the best proof of their efficacy. "Terrestrial" medicine has received a number of practical recommendations as a result of the study of weightlessness, which pertain, for example, to the problem of eliminating the results of man's long-term immobilization under clinical (and not only

clinical) conditions. Some interesting data were obtained from a study of the effects of weightlessness on the cardiovascular, respiratory, digestive, nervous and endocrine systems, system of immune defense and metabolic processes.

The first phase of the "attack on weightlessness" yielded some results, for which USSR State Prizes were awarded.

During implementation of the general program for space exploration, major advances were made with respect to the problem of screening cosmonaut candidates, i.e., the problem of healthy man, to whom representatives of "terrestrial" medicine devoted considerably less attention, for a number of reasons, than to sick man, although it was already apparent in the distant past that any pathology in some form or other is a manifestation of the functional patterns of healthy man. C. Bernard had already maintained that "... the conditions under which diseases develop cannot fail to impart strength to an organism that was not inherent in it prior to the disease or to develop pathological physiology distinct from normal physiology."<sup>3</sup>

I. V. Davydovskiy stressed the thesis that it is basically impossible to separate biological phenomena into physiological and pathological ones, since both are referable to natural history, i.e., they are "legitimate" in their biological substance. He maintains that "... the differences between physiology and pathology are attributable more to man's need to differentiate and classify natural phenomena.... Physiology and pathology that are separate in principle are ontological abstractions, the product of 'external thinking,' rather than the product of the dialectical method of cognition."<sup>4</sup>

This view directs the attention of physicians, as representatives of the science dealing with disease, to the fullest conception of a healthy body, since it is expressly in the laws of its functions that we solve the mystery of pathological phenomena. Interestingly enough, N. P. Ogarev, a man who had no connection with medicine, wrote in one of his letters to A. I. Gertsen: "... physiology and pathology coincide, and pathological phenomena develop in accordance with physiological laws; nature does not know about our scientific subdivisions, invented ... actually for our convenience, so that we would not become too confused."<sup>5</sup>

Space medicine breathed real life into these ideas, having demonstrated once more that the problem of healthy man is the key problem of general medicine, and it provided material to specialists indicative of this fact.

The problem of the norm, i.e., the aggregate of parameters that are inherent in the usual state of essentially healthy man with the usual (in relation to numerous averaged data) level of work capacity, is directly related to the problem of healthy man. Studies were begun at the Institute of Biomedical Problems, USSR Ministry of Health (IMBP) of the biorhythmological norms, since expressly these parameters react the most to negative environmental factors, and this was confirmed in recent studies of the effects of toxic factors on healthy man. In particular, it was shown, with the use of biorhythmological methods, that, following demonstrable normalization of general condition 27 h after intake of alcohol, worsening was observed after 45 h, then again, after



51 h (3d day after intake) normalization of this parameter was observed. These facts convincingly confirm the importance of data characterizing the norm.<sup>6</sup>

The novelty and complexity of problems related to space exploration caused new scientific directions to emerge. There was rapid and broad development of space biorhythmology. Within this direction, the problem of organizing daily work and rest schedules for cosmonauts was resolved. Elaboration of the guidelines for such scheduling required combined efforts to establish specific daily schedules for spaceflight participants and to determine the patterns of endogenous organization of biological cycles in the human body, the fluctuating nature of which was common knowledge. However, use of the conception of variability of vital functions turned out to be productive only in answering the question of motivating causes, the sources of this phenomenon. Such an answer was found as a result of in-depth theoretical research, and it permitted formulation of a definition of biological rhythm, according to which rhythm is a form of motion of living matter, in which there is objectivization of unity and struggle between mutually exclusive opposites of destruction and creation underlying the self-reproduction process. Further developments in the same direction established the leading role of the circadian rhythm in the general fluctuating system of the body, marked by signs of universality and necessity, i.e., the mandatory attributes of the law. The law of circadian rhythmicity makes it possible to define the forms of correlation between circadian rhythms, homeostasis and adaptation, and to come closer to comprehension of the desynchronosis phenomenon, disruption of coordination of circadian rhythms that is associated with any adversity striking any complex living system. The results of comprehensive analysis revealed that desynchronosis is a mandatory element, and the earliest one at that, of the general adaptation syndrome and that there is every reason to ascribe to it the rank of a central problem of space biorhythmology.

In the course of working on problems of space biorhythmology, conceptions were formulated and validated concerning the fluctuation of the adaptation process, constancy of circadian rhythms (consideration of which is important to the system of professional screening of individuals in critical occupations and, consequently, cosmonauts), guidelines of chronodiagnostics and biorhythmological distinctions of shift work. The periodically arising need (at a given stage of cosmonautics) to investigate schedules of work in shifts resulted in inclusion of questions of rules for shift work in the sphere of space biorhythmology. Work on these problems established the distinctions of the effect of night shift work on young healthy people, such as the phenomenon of "shift imprint," unification of course of circadian rhythm, use of which made it possible to intensify monitoring of individuals adjusting to unaccustomed sleep and waking schedules. Data obtained from the study of shift-work problems are acquiring special importance with the restructuring of the national economy, which determined the increasing role of shift work which, in turn, increased the amount of equipment and its profitability.

At the present time, intensive work is being pursued along the lines of defining the system of professional screening of cosmonauts, improving the monitoring of cosmonauts at different stages of flight and in the postflight period. Much attention is being devoted to medical monitoring of man during orbital flights. The system of telemetric recording of parameters of current status of the organism is being refined, and the telemetry system being used

in space is being used with success on the ground. The increase in technical equipment of space medicine has a beneficial effect on an analogous process in earth-bound medicine. Development of an instrument for measurement of partial oxygen tension in capillary blood during spaceflights by specialists of the USSR and GDR is one of the many specific examples of such an effect. This instrument is offering vast opportunities to intensive medical care, sports medicine, industrial medicine, functional diagnostics and other branches of medicine. On the example of this instrument, we see the effectiveness of international collaboration, primarily with socialist countries. At the present time, such collaboration through the Intercosmos program is broadening constantly, involving the progressive scientific manpower of capitalistic countries as well in the cause of space exploration for peaceful purposes.

However, the significant advances made in Soviet space medicine, their broad recognition and high praise cannot obscure the omissions and oversights that exist along the route of its progress or minimize the importance of restructuring thinking in both work on problems already set forth and the new ones that continuously arise.

In this aspect, the problem of correlation between content and form of scientific research in the area of space biology and medicine merits special attention.

The often used procedure of scientific research implies separation of theoretical and practical stages, prior scrutiny by the executor of a scientific project of its theoretical premises. Data obtained in the course of a study are, so to speak, "inscribed" in the structure of an adopted scientific paradigm. Separation of science into theoretical and practical areas was formed on expressly such a basis, and this was, in particular, reflected in the establishment of independent scientific institutions (institutes) of the sector type intended for work on strictly practical problems. In such institutions work on fundamental problems was completely (or almost completely) excluded and even viewed as some form of sedition. A formalized system emerged for scientific cognition proceeding, in accordance with dialectics, from vital contemplation to abstract thinking and from the latter to practice. Formalization was manifested by recognition of the separateness of these stages and, consequently, rejection or obscurement of the reality of their interpenetration.

The possibility and, in a number of instances, necessity of predominant orientation of research toward practical or basic problems by no means signifies that a "veto" is imposed on combining these routes or that they are indeed separated in the living actuality of scientific progress. It all depends on the concrete conditions of a concrete investigation. Such separation, which is warranted to some extent or other in a particular actual situation, may turn out to be an inhibitory factor along the way toward achieving results in another, equally real situation. Truth is always concrete. This general dialectical thesis has a direct bearing on the problem of correlation between fundamental and practical elements of scientific research.

The idea that there must be a close correlation between theory and practice has been repeatedly voiced in different forms. "A rift between ... theory and life, between theory and practice," remarked P. L. Kapitsa, "is a symptom of serious disturbances in normal development of science.... Harmonious development of theory and practice is absolutely mandatory in all branches of natural science."<sup>7</sup> "Science is unique: there are not two sciences ... for ages, science has been compared to a branching tree ... each branch of the tree ... has its own originality ... but the aggregate of branches belongs to the same whole, living plant--a tree."<sup>8</sup>

The researcher cannot limit himself to empirical thinking, i.e., thinking that stops before a phenomenon, since in actual scientific research such a halt is simply impossible, and a genuine researcher is not only and not so much a contemplator as he is an observer. And observation is not merely a photograph of a phenomenon, but its analysis, thinking about it, i.e., interpretive perception in which the following questions are always present: Why? How? The route of cognition is always a movement from a phenomenon to substance, and this route was laid by man's very need for creativity, for learning about the unknown, the new. For expressly this reason, empiricism and theorizing are inseparable from one another. "Accumulation of facts and delving deep into meaning by no means contradict one another," wrote A. I. Gertsen.<sup>9</sup> Moreover, a phenomenon as the arbitrary start of a scientific quest is not merely the sensory aspect of substance that can be removed, like a shell, from an object, alien to it in its substantiveness, since in reality ... "substance appears. A phenomenon is tangible."<sup>10</sup> In any object of research, as in some graphically represented phenomenon, there is a reflection of substance, and it is expressly through the direct contact with facts and their connection into a chain of successive links of organized perception that observation provides for continuity of motion "...from phenomenon to substance, from, so to speak, first-order substance to second-order substance, and so on unendingly."<sup>11</sup>

Solving urgent scientific problems in the absence of predeveloped theory does not imply use of traditional routes: transmission of these problems to some scientific centers concerned with investigation of basic problems (in particular, the Academy of Sciences), from which data come that guide the course of practical developments. True, ordinary thinking defends (often quite aggressively) such a route of investigations, regardless of concrete conditions under which they are pursued. This virtually inhibits scientific progress, and this is particularly dangerous when solving complicated and new problems.

Expressly such a situation is inherent in cosmonautics as a whole and in space medicine and biology in particular. In these disciplines, the solving process merges for both tactical and strategic problems, since separating them into successive phases would slow down implementation of the overall program of space conquest. The burning, urgent need to obtain answers to basic question that practice had already encountered with the first launches of manned spacecraft compelled representatives of space medicine to boldly search for the means of answering them, rather than shifting this burden to the shoulders of academy institutions. In fact, this was a restructuring of scientific thinking inherent in sectorial institutes, since this required, as M. S. Gorbachev stated at the June 1987 Plenum of the CPSU Central Committee,



a genuine combination of profound formulation of basic research and increased effectiveness of concrete developments, rather than a declarative combination.

Development of guidelines for organizing the optimum work and rest schedule for cosmonauts is an example of such solution to concrete problems of space medicine. For several years, there were two views about solving this problem: one that ascribed a conditioned reflex origin to circadian rhythms and the other that maintained that they were endogenous (programmed by nature). This struggle reflected the status of the matter in the area of basic problems of biorhythmology, where the "exogenous" and "endogenous" conceptions of circadian rhythms also competed. Expressly the former initially defined the guidelines for organizing the daily schedule aboard spacecraft.

In order to settle the question of optimum variant of 24-h schedule for cosmonauts, experimental and theoretical studies were pursued at the IMBP, USSR Ministry of Health, the results of which permitted formulation of a definition of circadian rhythm, establishment of its endogenicity and approval of the optimum sleep and work schedule for man in space. A difficult basic problem was solved, thereby enriching practice. From the very beginning of the space age, there emerged acutely the problem of time in the aspect of its practical significance, practical form of involvement in biomedical and psychophysiological processes.

The importance of considering time in the structure of these processes is common knowledge. But this recognition per se does not yet signify that the principles of timing concrete vital processes, i.e., planning that is placed on the fabric of time, are indeed included in setting up and conducting scientific investigations. Wherever there is such inclusion, a significant practical achievement emerges. This could be best illustrated by the establishment of a schedule for life on aircraft, which is based on the law of circadian rhythms and, consequently, the law of endogenicity of these rhythms. Another example is the professional screening of candidates for the so-called critical professions, i.e., those in which the workers are subject to significant tension, mainly neuropsychological, which usually is prolonged, exceeding the circadian cycles (24-h periods).

When screening candidates for such occupations (which include cosmonauts), exceptional importance is attributed to determination of resistance to extreme loads at any time of day. It is known that the level of such resistance is not the same within a 24-h cycle in different people, and that high daytime parameters of general physical condition (including readiness to work) do not always correspond (most often do not correspond) to equally high parameters (or even good and satisfactory) at other times. For this reason, individuals with high ratings who were screening during the usual daytime hours of commission work (daytime, most often mornings) are found, in a number of instances, to be incapable to exhibit the required level of professional skill at other times of day (late evening, particularly at night). This fact is very important. A thesis was formulated on the basis of such facts concerning functional chronodiagnostics--determination of resistance by means of functional load tests at different times of day (including nights), which no doubt has a direct bearing on improving the effectiveness of professional screening. However, repeated attempts to introduce to the practice of

special professional screening of the principles of functional chronodiagnostics did not succeed. Yet a cosmonaut, whatever schedule he adheres to, is still expressly a cosmonaut who must be in full possession of his professional skill at any time of day. The programs of spaceflights do not preclude sporadic involvement in work of any participant of any mission at different times of day. There is still a possibility and need for shift work aboard spacecraft, both in orbit and, in the future, during interplanetary flights. There is no necessity to speak of the importance of a high degree of readiness to work of cosmonauts, when even the slightest decline of professional skill could be the cause of a serious outcome. Coincidence of the time of an accident with the period of individually low constitutional resistance is fraught with expressly such a danger.

We should add to this that the physical condition and adaptability change under the effect of stress factors in accordance with the general biological law of fluctuation, complex periodicity of the process of adaptation to an environment. This law attributes the fluctuations in constitutional resistance to the times of its demonstrable decline.

Both the most pragmatic, routine concerns of science and its global problems emerge in a different aspect when scrutinized through the prism of time. Such orientation requires alteration of thinking, inclusion of the time factor as a mandatory component in its structure. This is a particularly pressing requirement for space medicine.

In cosmonautics, it is also important to provide spiritual comfort for all participants in a spaceflight for its duration. This problem comprises many special questions: guidelines and methodological implementation of psychological screening of cosmonaut candidates, their compatibility and cooperation in isolation and the closed environment of actual missions, leisure (particularly in the case of long-term flights), increased psychological stability in extreme situations, in particular, by means of special training in the preflight period with due consideration of possible transformation of general personality sets, interests, tendencies, which is possible in the course of a long-term flight.

The problem of synchronization of theoretical and practical (applied) directions of investigation, just like the problem of constructing them in the time aspect, acquires special importance in both general and, particularly, space psychology. While making a distinction between the theoretical and applied approach to scientific research is somehow justified with respect to other sciences, it is not acceptable with respect to the complex phenomena of man's spiritual life. Space psychology is faced with difficult problems, the solution of which requires, first of all, increasing the theoretical armamentarium for investigating the spiritual world of cosmonauts, in-depth analysis of their emotional experiences, consistent with space mission conditions. These problems are, thus far, a matter for the future development of space psychology. At the present stage of space exploration, the data of general psychology (and several of its already developed directions--industrial psychology, etc.) help solve routine problems of cosmonautics: programs developed on the ground have been implemented in past missions, the cosmonauts' general mental status remained on the level of criteria used in ordinary (ground-based) studies. All

this does not, however, eliminate the need for in-depth analysis of the actual structure of man's inner world when he finds himself under new conditions, known to him before only from descriptions and having no identical analogues on earth.

Some interesting results were obtained in studies pursued by clinicians, hygienists, immunologists and other specialists working in cosmonautics. All of them are used not only in cosmonautics, but other branches of "terrestrial" health care.

Development of Soviet cosmonautics has resulted in considerable achievements referable to many disciplines, including biology and medicine, which originated new scientific directions. Numerous important problems have been formulated and solved; results of broad scientific relevance have been obtained and, at the same time, new difficult problems have been advanced, which require bold overcoming of many dogmas reinforced by tradition, restructuring of scientific thinking. The real achievements of such restructuring will be the contribution of space medicine to the advances, with which our country is celebrating the 70th anniversary of the Great October Socialist Revolution.

#### FOOTNOTES

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2. P. Sharden, "Fenomen cheloveka" [Phenomenon of Man], Moscow, Progress, 1965.
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4. Ibid.
5. N. P. Ogarev, "Izbrannyye proizvedeniye" [Selected Works], Moscow, Gosizdat khudozhestvennoy literatury, 1956, Vol 2, p 539.
6. V. P. Latenkov and G. D. Gubin, "Bioritmy i alkohol" [Biorhythms and Alcohol], Novosibirsk, Nauka, 1987, p 172.
7. P. L. Kapitsa, "Eksperiment, teoriya, praktika" [Experimentation, Theory and Practice], Moscow, Nauka, 1977, p 35.
8. A. I. Gertsen, "Izbrannyye filosofskiye proizvedeniya" [Selected Philosophical Works], Moscow, OGIZ [Association of State Publishing Houses], 1948, Vol 4, p 100.
9. Idem, Ibid, 1948, Vol 4, p 110.
10. V. I. Lenin, "Poln. sobr. soch." [complete Works], Vol 29, p 227.
11. Ibid.

## RESULTS OF LONG-TERM ELECTROCARDIOGRAPHIC MONITORING OF COSMONAUTS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (manuscript received 9 Mar 87) pp 10-14

[Article by Ye. M. Romanov, N. P. Artamonova, Z. A. Golubchikova, A. F. Zavadovskiy, M. M. Korotayev, V. R. Lyamin and V. D. Turbasov]

[English abstract from source] This paper is the first summary of regular electrocardiographic examinations of 21 cosmonauts who made 42 space flights during the years 1964 through 1985. Electrocardiographic examinations were performed before, during and after flight. The most common ECG abnormality recorded was extrasystolic arrhythmia that occurred very frequently during provocative tests. In long-term flights changes in the phase of ventricular repolarization were predominant due to the effects of microgravity and other flight factors. The ECG changes were transient and of insignificant prognostic value. Long after return to earth three cosmonauts developed ECG changes typical of myocardial infarction, hypertension or hypertension combined with latent ischemic heart disease. It is maintained that these pathologies were not caused by the exposure to space flight effects.

[Text] Heretofore, there were no reports in the literature accessible to us concerning the results of successive ECG examinations performed on cosmonauts throughout the period of their training for spaceflights (SF), during flights and for a long postflight period. Extrasystoles, changes in waves and vectors of QRS and T, as well as transient changes in atrioventricular and intraventricular conduction time were noted in some cosmonauts who had flown in craft of the Voskhod and Soyuz type, and the Salyut-6 orbital station [1-4, 6-9]. The baseline ECG and vectorcardiographic (VCG) parameters were restored up to 1 month after long-term missions [6]. According to the data of American authors, extrasystoles, which were occasionally multiple or in groups, were a frequent type of change in astronauts who participated in the Apollo, Skylab and Space Shuttle programs, and in 1 astronaut this was observed in the form of brief bigeminy [11]. More serious rhythm disturbances (short-term atrioventricular rhythm and atrioventricular block) were also observed [13]. VCG parameters reverted to preflight values within 5-10 days after longer missions (up to 84 days). Extrasystole was observed in 1 cosmonaut on the 21st postflight day, which the authors interpreted as



being unrelated to the flight [13, 14]. Another cosmonaut had a myocardial infarction 1.5-2 years after SF, the cause of which could have been coronary vessel sclerosis that was not detected before the flight [12].

In the opinion of American and Soviet researchers, the changes in bioelectric activity of the heart are attributable to hypokalemia [11], changes in blood volume in chambers of the heart, deconditioning of the cardiovascular system [13], alteration of extracardiac regulation, changes in electrolyte metabolism, intracardiac hemodynamics, autonomic imbalance, as well as possibly metabolic changes in the myocardium [3, 6, 7]. It should be noted that the changes in bioelectric activity of the heart during flights were not clinically significant [3, 6, 13].

Methods ECG studies were performed on 21 cosmonauts who had made 42 flights from 1964 through 1985. One of them had been under medical observation for up to 5 years, 5 were under observation for 6 to 10 years, 5 for 11 to 15 years and 10 for 16 to 20 years. Seven cosmonauts each had participated in missions one, twice and 3 times.

The ECG (in conventional leads and D-S) was recorded before, during and after the missions using Mingograph, Polynom-2M, Aelita-01, Alpha and Beta equipment, both at rest and during functional load tests (postural, LBNP [lower body negative pressure], graded exercise on a cycle ergometer, testing in pressure chamber and on a centrifuge). In addition, there was ECG monitoring during the powered stages of flight, during performance of dynamic operations and extravehicular activity. The number of preflight and postflight examinations varied, depending on duration of training and recovery periods, and in the case of long-term SF they were performed once every 2-3 weeks. The ECG was analyzed using conventional methods [5, 9].

## Results and Discussion

Analysis of the studies revealed that the ECG was normal at all stages of observation in three cosmonauts. All of them had made short-term flights: two of them once each and one, twice. Changes were observed on the ECG of the other 18 cosmonauts (see Table). We did not take into consideration tachycardia at lift-off, insertion and descent to earth, since it was inherent in all cosmonauts. Marked bradycardia (33-42/min) was observed at all stages in only one cosmonaut, and it was apparently the individual norm and did not affect his work capacity or tolerance to functional loads. Marked preflight sinus arrhythmia of the respiratory type ( $\Delta HR$  [heart rate]  $\geq 25$ /min) was noted in two individuals, and it persisted as well in flight and after returning to earth.

Migration of source of rhythm from the sinus node to the mid right-atrial level was observed in two people. In one case, it appeared in the recovery period following a load test while training for the mission and in the readaptation period, and in the other only in the recovery period. Most probably, the migrating rhythm appeared as a result of unbalanced neuro-vegetative regulation of cardiac function.

ECG changes in cosmonauts before, during and after flights  
(number of cases)

ECG changes	Preflight		Inflight		Postflight	
	at rest	function. tests	at rest, dynamic operations, EVA	function. tests	at	function. tests
Dysrhythmias:						
sinus bradycardia (HR <40/min)	1	—	1	—	1	—
sinus arrhythmia ( $\Delta$ HR >25/min)	2	—	2	—	2	—
migration of rhythm source to atria	—	1	—	—	2	—
extrasystole	2	14	8	1	2	12
Increased duration of: atrioventricular conduction (P-Q interval >0.20 s)	1	—	—	—	—	—
intraventricular conduction (QRS complex >0.10 s)	1	—	1	—	—	—
partial block of right branch of His' bundle	1	1	1	1	1	1
Change in de- and re-polarization phases:						
decreased amplitude of QRS waves	—	—	11	—	11	—
decline and flattening of T waves	—	—	14	—	18	—
deformity of T waves	—	5	2	—	1	—
horizontal depression of S-T segment to 0.2 mV	—	—	—	—	1	2
Increase in electrical activity of:						
right ventricle	—	—	—	—	2	—
left ventricle	—	—	—	—	2	—

The most frequent rhythm disorder in cosmonauts was appearance of extrasystoles. They were demonstrated for a total of 39 times in 13 people. Their supraventricular localization prevailed somewhat over ventricular (23 and 16 times, respectively). A monotonopic extrasystole was found 38 times, polytopic, once. As a rule, they were demonstrable in the form of single extrasystoles (35 times), and less often in groups or pairs (4 times).

During and after the preflight training periods, extrasystoles at rest were demonstrated much less often (4 times) than during functional tests (26 times).



It should be noted that resting extrasystoles during SF were observed, on the contrary, more often than with loads. Extrasystole in flight was usually observed during insertion of the spacecraft into orbit or in the first orbits around the earth, i.e., during the period of acute adaptation to weightlessness. In only one instance was it observed during EVA [extra-vehicular activity] (2 single supraventricular extrasystoles). Examination immediately after the flight revealed single supraventricular extrasystoles that disappeared after the 1st month of recovery in two people.

With use of load tests, extrasystole appeared most often during exposure to accelerations on the centrifuge (18 times in 11 people). It was observed less often during the exercise test (7 times in 6 people in the training period), once when tested in the pressure chamber. In one cosmonaut, extrasystole was demonstrable at all stages of observation in the cycle ergometer test. It was polytopic after the second flight. Subsequent 8-year observation failed to reveal extrasystole, and it is only during a third short-term flight that three isolated extrasystoles were noted at the insertion stage.

During load tests, resting extrasystole did not elicit impairment of well-being, work capacity in any of the 13 cosmonauts, and it was not associated with any clinical manifestations, which was indicative of its functional origin.

During the period of preflight training, one cosmonaut showed transient fluctuation of P-Q interval from 0.20 to 0.22 s at rest for 1 week, but this never recurred thereafter. Perhaps, the brief increase in atrioventricular conduction was related to vegetative-humoral changes following head colds.

In the preflight period, one subject periodically presented with a slight increase in duration of WRS complex from 0.10 to 0.12 s without shortening of P-Q interval or deformity of the ventricular complex. During a long-term SF and for the next 5-year observation period such changes or any others were not demonstrable on the ECG. In another cosmonaut, an increase in intraventricular conduction from 0.10 to 0.12 s was observed only during a short-term flight, and it was associated with shortening of P-Q interval from 0.14-0.15 to 0.12 s without change in heart rate or configuration of WRS complex, which confirms the functional nature of the demonstrated changes. Regular follow-up on this cosmonaut for 10 years after SF failed to reveal any disturbances in intraventricular conduction at rest. During the cycle ergometer test, performed both before and after SF, he demonstrated supraventricular extrasystoles, and in the last 3 years, at the age of 47-50 years, an ischemic type of ECG reaction to exercise appeared. In the same period, there was distinct demonstration of clinical symptoms of atherosclerosis, including impairment of lipid metabolism.

During the screening process, a partial block of the right branch of the bundle of His was found in one cosmonaut; it did not change for 10 years, which included 2 long-term flights.

In the course of long-term observation of cosmonauts no appreciable changes in the QRS complex were demonstrable.

A 10-25% decrease in amplitude of R and S waves, mainly in the left thoracic leads, was observed in 11 cosmonauts during long-term missions, 2-3 months after they began. On the first postflight days, this decline persisted and even increased somewhat, probably due to tachycardia, then it gradually disappeared within 2-4 weeks. While virtually no repolarization phase changes were demonstrable, or else only slight decrease in amplitude of T waves (3 times in 3 people) was observed in short-term flights, in the case of long-term SF, all 11 cosmonauts showed a distinct diffuse decline starting in the 2d-3d month of the mission. The process of change in repolarization phase prevailed over changes in myocardial depolarization phase (decrease in amplitude of some waves and in general in voltage of WRS complex), which lead to increase of R/T. As a rule, the amplitude of T waves showed 25-50% decrease, as compared to preflight values. Dual-peak deformity of T waves was sporadically observed in 5 cosmonauts at rest or during load tests in the training period and in 2 others during long-term SF. In one of these cases, there was some increase in deformity of T waves in the early postflight days. The inflight changes in T waves did not reach the range of pathological changes [5, 9].

The reported changes in bioelectrical activity of the heart during long-term SF are apparently attributable to the longer effect of weightlessness, reflex alteration of hemodynamics, change in fluid-electrolyte metabolism and microcirculation.

On the first postflight day, most cosmonauts presented with varying degrees of sinus tachycardia. There was a corresponding increase in voltage of P waves and decrease in WRS complexes and T waves. In one individual, a horizontal shift of S-T segment to 0.2 mV appeared immediately after the spaceflight. In two cosmonauts, we found an increase in electrical activity of the right ventricle, which can apparently be attributed to rotation of the heart with the right ventricle to the front after returning to earth's gravity. After 2 weeks of exposure to earth's gravity, 2 other individuals presented with signs of increased electrical activity of the left ventricle, which coincided with the increase in motor activity.

ECG parameters recovered within 1-2 weeks after relatively brief SF, whereas 1-2.5 months elapsed following long-term flights before recovery took place. Total recovery of baseline ECG, even after very lengthy (up to 237 days) flights is indicative of the functional nature of the changes. Horizontal shift of S-T interval below the isoelectric line to 0.2 mV was noted in one cosmonaut 3 and 17 years after SF, during a test with graded exercise. Atherosclerosis was subsequently diagnosed in this individual.

Prolonged and regular medical observation made it possible to detect development of cardiac pathology in three cosmonauts. In one of these cases, large-focus myocardial infarction developed 2 years after his third short-term flight, at the age of 49 years. In the second cosmonaut, who had made 3 short flights, the diagnosis of essential hypertension was made 4 years after the last mission, at the age of 52 years; this was confirmed by appearance of signs of left ventricular hypertrophy on the ECG. His tolerance to functional tests was good. In the third cosmonaut, the diagnosis of essential hypertension and latent form of ischemic heart disease was made at the age of 56 years,

17 years after his only short-term mission. The resting ECG showed distinct signs of hypertrophy of the left ventricle and horizontal depression of the S-T segment in the range of 0.05-0.1 mV, and during load tests it was 0.2-0.25 mV.

The long interval between completion of the last flights, when cosmonauts had no pathological changes, and detection of changes in physical condition enables us to exclude a causative link between spaceflights and these diseases.

Thus, the foregoing enables us to maintain that the observed ECG changes in cosmonauts were functional in nature, and they disappeared upon elimination of their causes. The sporadic appearance of extrasystole in most cosmonauts often coincided with development of situationally caused psychoemotional and physical tension during the medical examination with use of special load tests, and it had no serious prognostic significance. ECG changes appeared at the long term following spaceflights, which confirmed the clinical diagnosis, in only three people in the presence of development of cardiac pathology.

Comparative analysis of the results of long-term ECG studies of a group of Soviet and American cosmonauts revealed that extrasystolic arrhythmia is one of the most frequently encountered changes. At the same time, the functional disturbances in the atrioventricular system, which were demonstrated during flight by American researchers in a few cosmonauts, were not found in Soviet ones. Evidently, these differences are attributable to individual distinctions of cosmonauts.

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IMPROVEMENT OF HUMAN TOLERANCE TO HEAD-PELVIS (+Gz) ACCELERATIONS BY  
INCREASING HYDRATION

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21,  
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[Article by A. R. Kotovskaya, S. Baranski, D. Gembizka, M. Voitkowjak,  
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[English abstract from source] Experiments were performed to determine tolerance to head-to-feet (+Gz) acceleration in 62 test subjects aged 23 to 33 years. They were rotated in a human centrifuge before and after they consumed water and water-salt supplements under the conditions of normal activity or dry immersion simulating microgravity effects. During the centrifugation experiments the following parameters were recorded: stroke volume and cardiac output, arterial pressure by means of Korotkov sounds, electrolytes, total protein and hematocrit. Water and water-salt supplements were found to produce a beneficial effect on acceleration tolerance: tolerance threshold increased, stability of cardiorespiratory functions grew, cardiac arrhythmias developed less frequently. The efficacy of the methods increasing the hydration level was related to the amount of water consumed and retained in the body. It is recommended to use a differential approach to the development of procedures for increasing body hydration to be employed in aerospace medicine.

[Text] It is known that dehydration of the body, or more precisely a decrease in circulating blood volume, has an adverse effect on tolerance to any factors related to redistribution of blood in the direction of the lower part of the body.

There are descriptions in the literature of the adverse consequences of dehydration of diverse genesis, with respect to orthostatic stability [8], tolerance to lower body negative pressure (LBNP) [7] and +Gz accelerations [5].

At the same time, it is known that the effect of prolonged longitudinal accelerations per se can lead to 6-8% decrease in circulating blood volume due to filtration of plasma into the extravascular space [6].



For this reason, artificial elevation of hydration level in the body can be viewed as a pathogenetically justified means of increasing human tolerance to +Gz accelerations, in order to counteract the effects of hypohydration.

According to data in the literature, when functional change is unchanged, re-hydration alleviates somewhat the function of the cardiovascular system when exposed to accelerations without raising the tolerance threshold [2].

Our objective here was to further investigate the efficacy of water-salt and water supplements in enhancing human tolerance to accelerations in the head--pelvis direction (+Gz), as well as to define the possible areas of application of such supplements in aerospace medicine.

#### Methods

PNR [Polish People's Republic] and USSR centrifuges were used in the studies, which were pursued on 62 people 23 to 33 years of age.

The subjects were submitted to +Gz accelerations for as long as they could tolerate them. Centrifuge rotation was stopped when subjects developed visual disorders or their precursors (diminished amplitude of pulse to less than 25% of the baseline in earlobe vessels and drop of systolic arterial pressure--BP<sub>s</sub>--to 40 mm Hg or less in the same vessels), as well as upon appearance of arrhythmia in the form of polytopic, group or multiple extrasystoles [3, 4].

The studies were performed before (control group) and after intake of water supplement (WS) at the rate of 7 ml (1st group) and 14 ml (2d group) water per kg weight and following 3-4-fold intake of water and salt supplements (WSS) to the food allowance, at the rate of 18 ml water and 0.15 g sodium chloride/kg body weight (3d group). The WS was taken under usual living conditions, 30 min before rotation, while WSS was prescribed during simulation of weightlessness by means of 7-day "dry" immersion, in divided doses, 16 h before exposure to accelerations [2].

In these studies, we recorded the electrocardiogram (ECG) to detect dysrhythmia and calculate heart rate (HR); stroke volume (SV) and cardiac output (CO) by the method of thoracic tetrapolar rheography; systolic, diastolic (BP<sub>d</sub>) and pulse (BP<sub>p</sub>) arterial pressure in brachial vessels according to Korotkov sounds. For integral evaluation of the body's response to accelerations, we calculated the index of cardiovascular tension (CVT). CVT was calculated using the formula,  $\Delta HR - \Delta BP_s + \Delta BP_d$ , where each parameter was determined as the difference between its value before rotation and during rotation.

In addition, we assayed electrolytes, total blood protein and hematocrit, as well as fluid retention by the method of balance studies of fluid intake and output.

#### Results and Discussion

Intake of WS and WSS had a beneficial effect on tolerance to accelerations. The range of +Gz tolerance increased by 0.3 G in the 1st group of subjects,



and by 0.8 G in the 2d group, i.e., with statistical reliability ( $p < 0.01$ ). In the 3d group, tolerance to accelerations increased by 0.5 G as compared to the findings after 7-day immersion without preventive measures ( $p < 0.05$ ); however it did not reach the baseline level (with ordinary motor activity; Figure 1).

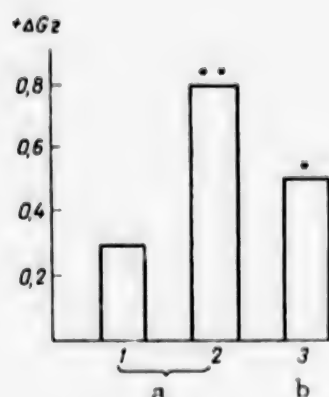


Figure 1.

Increase in tolerance to +Gz accelerations as a result of intake of supplemental amounts of fluid under ordinary conditions (a) and after simulated weightlessness (b)

1, 2) after intake of 7 and 14 ml/kg water, respectively

3) after intake of 18 ml water and 0.15 g sodium chloride/kg body weight

\* $p < 0.05$ , as compared to control

\*\* $p < 0.01$  " " " "

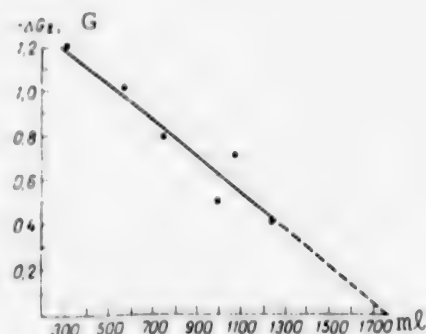


Figure 2.

Individual decline in tolerance to +Gz after simulated weightlessness as a function of amount of fluid retained after intake of WSS

X-axis, fluid retention (ml);

y-axis, ΔGz (G)

Subjectively, there was better tolerance to accelerations after fluid intake; the subjects showed no appearance of unpleasant or painful sensations in the region of the heart, which were inherent in the control tests. Nor did we demonstrate worsening of vision, which is so typical of postimmersion acceleration.

The response of the cardiovascular system to acceleration was better after use of WS and WSS than in the control. HR increment was 4.1% lower in the 1st group, 8.4% lower in the 2d ( $p < 0.05$ ) and 9% lower in the 3d.

After artificial rehydration (3d group), SV during rotation on the centrifuge held at a higher, more stable level and constituted  $54 \pm 3.1$  ml at 3 G, whereas in the postimmersion control studies it was  $39 \pm 4.3$  ml ( $p < 0.05$ ; Table 1). CO was 22% greater at 3 G following intake of WSS, and it was maintained due to stability of systolic expulsion, rather than at the price of maximum increase in HR.

Dysrhythmia appeared less often (by 7%) than in the control studies, and in all instances amounted to a solitary extrasystole.

The dynamics of the CVT index indicated that there was considerable decrease in functional strain on the cardiovascular system under the effect of +Gz

( $p < 0.05$ ; see Table 1) at 3 G. However, according to the recorded values for the CVT index, the subjects tolerated accelerations after simulated weightlessness worse than with unchanged functional state of the body.

Table 1.

Dynamics of cardiovascular system parameters ( $M \pm m$ ) with exposure to +3Gz accelerations following immersion without preventive measures (A) and with use of WSS (B)

Parameter	Series	Base-line	3Gz
SV, m	A	70±6.6	39±4.3
	B	76±12	54±3.1*
CO, /min	A	6.0±0.7	6.5±0.4
	B	6.4±0.8	7.9±0.5
HR/min	A	85±4.1	165±4.0
	B	85±5.8	156±7.3
BP <sub>p</sub> , mm Hg	A	60±3.7	59±2.9
	B	56±4.0	77±12.1
CVT index	A	—	81±7.9
	B	—	52±10.6*

\*  $p < 0.05$ .

During rotation we observed significant elevation of BP<sub>p</sub>, on the average from 56 to 77 mm Hg at 3 G, whereas under control conditions this parameter held at a level of 58-60 mm Hg.

The beneficial effect of intake of excessive fluid was apparently related to increase in circulating blood volume. This was indicated by the reliable ( $p < 0.01$ ) decline of electrolyte (sodium and potassium) levels and hematocrit, as well as relative increase in plasma volume (Table 2).

At the same time, we recorded a 4% decrease in plasma volume, corresponding to findings described in the literature [6], in the control group of subjects who did not take fluids prior to rotation on the centrifuge.

Table 2. Dynamics of biochemical parameters of blood before start of rotation and intake of WS, and after exposure to accelerations

Parameter	Group of subjects	Before rotation on centrifuge	After rotation on centrif.	$\Delta$ , %
Osmotic pressure, mOsm/l	1	286±2	292±5**	+2.1
	2	290±5	294±5*	+1.38
	3	288±4	285±4*	-1.04
Total protein, g/l	1	69.01±8.83	73.75±8.50**	+6.87
	2	75.07±5.57	79.69±3.18**	+6.15
	3	80.85±8.57	85.39±8.09	+5.61
Hematocrit, l/l	1	0.473±0.023	0.485±0.022**	+2.54
	2	0.481±0.022	0.477±0.020*	-0.83
	3	0.486±0.021	0.472±0.023*	-2.88
Hemoglobin, mmol/l	1	10.38±0.51	10.63±0.49**	+2.4
	2	10.56±0.43	10.51±0.46	-0.47
	3	10.66±0.48	10.37±0.50	-2.72
Relative change in plasma volume*	1	—	—	-4.15
	2	—	—	-1.25
	3	—	—	-5.24
Na <sup>+</sup> ions	1	140.3±2.1	141.1±2.0	+0.57
	2	138.6±1.4	138.0±1.8	-0.43
	3	140.1±1.9	138.6±1.4**	-1.07
K <sup>+</sup> ions	1	4.62±0.58	4.27±0.48*	-7.57
	2	4.25±0.37	3.88±0.35**	-8.70
	3	4.57±0.47	4.04±0.39**	-11.60

\*  $p < 0.05$ .  
\*\*  $p < 0.01$ .

After intake of WS, a high hydration level persisted for about 40 min, whereas after intake of WSS it lasted for 8 h. After this time, excessive fluid was eliminated.

As shown by the results of our studies, the efficacy of agents that increase hydration level depended on the amount of water taken and volume of fluid retained.

Thus, after intake of WS in a dosage of 14 ml/kg body weight (2d group), the increase in range of tolerance to accelerations constituted 0.8 G and was reliable ( $p < 0.01$ ), whereas after intake of 7 ml fluid/kg (1st group), tolerance to accelerations increased by only 0.3 G, as compared to the control, i.e., it was not statistically significant (see Figure 1).

Testing of subjects in the 3d group established that individual decline in tolerance to +Gz accelerations following immersion was a function of amount of fluid retained. As can be seen in Figure 2, in spite of the standard protocol for WSS intake, fluid retention varied in the subjects, ranging from 310 to 1200 ml (mean  $818 \pm 140$  ml). The least decline in tolerance to accelerations (by 0.4 G) was noted in a subject, fluid retention in whom constituted 1200 ml, i.e., the maximum. Conversely, the subject who showed the least fluid retention (310 ml) demonstrated the most significant decline of acceleration tolerance following immersion--by 1.2 G. He also presented with persistent visual disorders.

On the whole, on the example of the 3d group, we found a close inverse correlation between amount of fluid retained and degree of decline in tolerance to accelerations, as well as a regression function described by the equation,  $y = 1.45 - 0.0008x$ , where  $y$  is the magnitude of decline of postimmersion tolerance to accelerations and  $x$  is the volume (in ml) of fluid retained in the body (see Figure 2). Coefficient of correlation  $r = -0.96$ .

As can be seen in Figure 2, in a concrete investigation, theoretically there must be about 1700 ml retention of fluid in order to prevent decline of tolerance to accelerations following simulated weightlessness.

Consequently, according to the data we obtained, a relative increase in fluid intake and retention enhances the efficacy of the means used. Use of WS and WSS combined with analogues of antidiuretic hormone is promising; as shown by A. I. Grigoryev et al. [1], this leads to retention of about 2.5 l fluid in the body and has a marked beneficial effect on orthostatic tolerance.

Thus, our findings are indicative of the desirability of using agents that increase body hydration in order to enhance tolerance to accelerations. It was established that a differentiated approach must be used to development of concrete methods of raising the hydration level in the practice of aerospace medicine.

Single intake of water prior to taking off may be found effective for purposes of aviation medicine. As applied to problems of space medicine, longer intake of water for many hours to saturate the human body with fluid and electrolytes is required prior to a planned descent of a spacecraft to earth.

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EFFECT OF SPACEFLIGHT FACTORS ON BIOLOGICAL MATERIAL FLOWN ABOARD COSMOS-1514  
BIOSATELLITE

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21,  
No 6, Nov-Dec 87 (manuscript received 24 Feb 87) pp 18-24

[Article by Y. Gaubin, M. Delpoux, J. Bionov, H. Planel, G. Gasset,  
B. Pianezzi, M. Burg and A. Barbast (France)]

[English abstract from source] The effect of space flight factors, particularly radiation, on *Artemia salina* cysts, tobacco and rice seeds (embryos and caryopses) was investigated. *Artemia salina* cysts showed no significant deviations in response to this exposure. Minor changes in certain cyst groups were related to their packing, humidity and preirradiation level. Tobacco seeds exhibited a higher frequency of somatic and morphological changes that were independent of their packing arrangement. Rice seeds developed no changes that could be associated with space flight effects. The changes seen are considered in relation to the radiation dose absorbed. The results obtained in different space experiments are discussed.

[Text] The purpose of the Bioblock-5 experiment performed aboard the artificial earth satellite, Cosmos-1514, was to investigate the effects of spaceflight factors (SFF), in particular cosmic radiation, on material at the latency period exposed to different levels of radiation.

In selecting the biological material, we proceeded from the technical distinctions of attaching the biological objects used in the Bioblock experiment [2-4], as well as the Biostack 1 and 2 experiments (flight aboard Apollo 16 and 17) [6, 7]. This method permits determination of the trajectory of heavy charged particles of cosmic radiation in relation to the biological material and demonstration of objects directly touched by these particles after the flight. As a result of exposure to SFF, we found differences between biological objects placed in a monolayer and loosely.

For this reason, in the Bioblock-5 experiment, we used two types of stowing biological objects. The purposes of our studies did not include investigation of the biological effect of individual heavy ions. We only examined the general effect of SFF, including cosmic radiation. Moreover, nuclear emulsions could not be used in the external container in open space.



In our experiment we used two types of biostacks. The first is the traditional bioassembly with biological layers (100×800 mm). It is stowed in a spacecraft under protective foil 3 to 5 g/cm<sup>2</sup> in thickness. A detailed diagram of the biostack is illustrated in Figure 1. It consists of 8 biological monolayers and 4 boxes with loose biological material, with thermoluminescent dosimeters. The monolayer consists of a metal macrofoil plate with holes, in each of which there is one biological object. Thus, a single biological layer contains about 5000 cysts. Two monolayers of *Artemia salina* and two layers of tobacco seeds were connected with nuclear emulsions I LFORD-K5, and the other monolayers were self-contained.

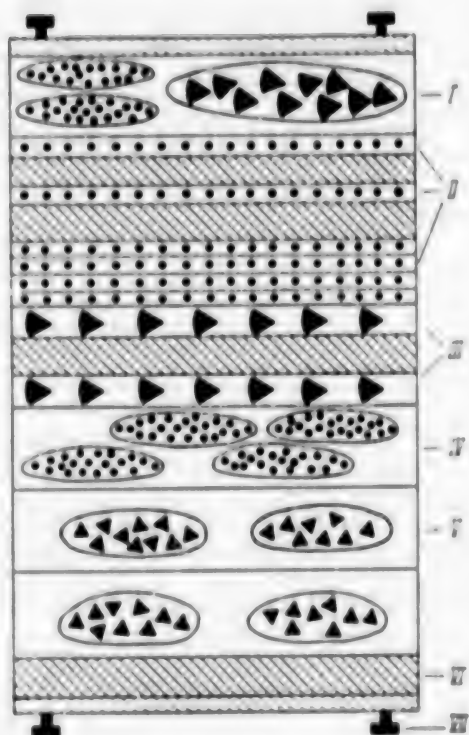


Figure 1.

Diagram of inner container biostack

- I) *Artemia* cysts and tobacco packed loosely
- II) monolayer of *Artemia* cysts
- III) monolayer of tobacco
- IV, V) *Artemia* cysts and rice, respectively, packed loosely
- VI) nuclear emulsions
- VII) metal rods

doses of radiation, depending on their location in the stack. Absorbed dose was determined using dosimeters (LiF). Dosimetry revealed that absorbed dose was the same in both the interior and exterior biostack with maximum shielding. In the exterior biostack, the dosage with minimal shielding was 0.15 to 0.037 Gy, depending on location of the detectors.

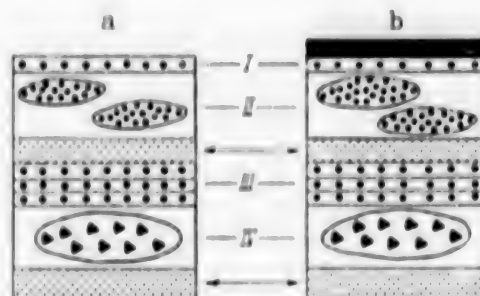


Figure 2.

Diagram of biostack in external container with different forms of shielding

- a) 0.0015 g/cm<sup>2</sup> shielding
- b) 0.35 g/cm<sup>2</sup> "

- I, II) *Artemia* cysts in monolayer and loose, respectively
- III) monolayer of *Artemia* cysts
- IV) loose tobacco

Arrows point to dosimeters

Figure 2 illustrates the composition of the biostack outside the spacecraft. The biological material, packed in a monolayer or loose in polyethylene bags was placed in 4 aluminum boxes (40 mm in diameter, 15 mm high) and covered on the top with a lid 0.0015 to 0.3 g/cm<sup>2</sup> thick. These packages were placed outside the spacecraft, where the biological material was exposed to different



Studies of *Artemia* cysts. We used eggs (cysts) of the liman [drowned river valley] shrimp at the embryonic stage of gastrula. They contain about 4000 cells 200  $\mu$ m in diameter. They are contained in a solid capsule and have only 3% water. Cysts can remain in this state for many years. They may again be capable of development after hydration. After the capsule breaks (nibbled through) and exit of the embryo (hatching) the cysts yield live nauplii. Adulthood is reached in 3 weeks. In the experiment, we used two types of cysts: with high and low hatching percentage. Some of them were dry (~3% water) or humid (68% water), in accordance with the method of T. Clegg [1]. Dry and hydrated cysts were pre-exposed to radiation in doses of 100 and 500 Gy.

We used two methods of cyst upkeep: in polyethylene sealed sacks (for the interior container) and closed, but unsealed bags (for the exterior container), as well as on a sheet of macrofoil (coated with gold) with 250- $\mu$ m holes. After the flight, the cysts were stored in a desiccator under controlled conditions. We took 50 cysts from each batch for examination and placed them in a medium with synthetic sea salt. After hatching, we added nutrient medium with *Dunaliella viridis* algae. From the moment they hatched, we studied the life span of 20 specimens from each batch. The larvae were numbered and fed daily. The study was limited to the first 20 days of life, the period that is the most sensitive to environmental factors. The following served as criteria for assessment of the biological effect: shells broken (%), amount hatched and live nauplii on the 4th day after start of hatching to sexual maturity. The results are means obtained for different groups. Margin of error was represented by a confidence interval ( $p = 0.05$ ). Data on mean life span were calculated by the method of dispersion analysis.

Studies of *Nicotiana tabacum* tobacco seeds. Tobacco seeds were chosen because they carry a genetic marker that permits detection of the mutagenic effect of environmental conditions under which seedlings and vegetating plants develop. The Bioblock-5 experimental conditions compelled us to work only with seeds.

The Xanthi variant of tobacco (*Nicotiana tabacum*) carries the following genetic marker:  $a_1^+/a_1^+a_2^+/a_2^+$ , it has two loci  $a_1$  and  $a_2$ , which control synthesis of chlorophyll. The heterozygotic structure of the system causes a partial deficiency in the chlorophyll that explains the greenish-yellow color of the leaves. Any genetic event that strikes one of the alleles is manifested by a change in chlorophyll synthesis and the color of the stricken cell changes. Its offspring has spots (somatic change) on a dark-green background. A count of the changes permits determination of the number of genetic changes manifested in the course of plant development.

Tobacco seeds are also a convenient model to use because they are very small (600  $\mu$ m long, 400  $\mu$ m wide). This enables us to place them on attachment grids and packages of a small size, which are then placed in wells where the biological material is in a loose state within or without the biostack.

The genetic effect was assessed by counting the incidence of somatic variations in the first two leaves normally formed from primordia on the embryo. In addition, we conducted a concurrent observation that enabled us to establish

that there are differences between the rate and percentage of germination in the experimental groups (control and flight). Occasionally, morphological abnormalities appear on the cotyledons and first leaflets formed from embryonic cells (growth points). Although these criteria do not correspond to the main purposes of studying tobacco, it is interesting to describe and consider these physiological and morphological changes which illustrate, in a number of instances, the effects of factors inherent in spaceflights.

Studies of isolated rice caryopses and embryos. Isolated embryos and caryopses were exposed to SFF for 5 days. We selected two varieties of rice for our studies: Tsigalon with large seeds that contained less albumin and Delta with long grains. Effects were assessed according to the following parameters: results of caryopsis microanalysis, dynamics of growth and length of seedlings after in vitro cultivation. In all instances, the results obtained on caryopses and embryos of flight and control groups were compared to one another.

After returning to earth, some of the caryopses and embryos were inoculated in test tubes on filter paper soaked in sterile, liquid nutrient medium (Miller's medium). Carbon (glucose at the rate of 10 g/l<sup>-1</sup>) was added to the medium for embryos. The sample was first sterilized in calcium hypochloride.

Cultivation was done at 25°C. Seedlings were exposed to artificial daylight (5000 lux) with photoperiodicity of 12 to 24 h.

## Results and Discussion

### Studies of Artemia Cysts

Interior container. The effects varied in dry cysts arranged in a monolayer, and they depended on the layers used in the biostack; there was significant inhibition of cysts with low percentage of development and some stimulation of cysts with high percentage of development (Table 1).

Table 1. Percentage of hatching of dry cysts arranged in a monolayer or loosely, with different levels of development

Variant	High development level		Low development level	
	monolayer	loose	monolayer	loose
Control	77,0 ± 3,69	86,80 ± 2,97	60,80 ± 4,28	70,10 ± 4,00
Flight	81,40 ± 3,41	83,80 ± 2,23	49,00 ± 4,38*	60,10 ± 4,38*

Note: Here and in Tables 2-4: asterisk indicates that differences between variants are reliable.

Reliable decrease in capacity for development was noted in dry cysts that were loosely arranged only in the layer with low percentage of cyst development. Conversely, life span did not change. For moistened cysts packed loosely no changes were demonstrable; this applies to cysts with high percentage of development of which there was only one in this variant. Significant stimulation was noted on the same cysts 3 months after the first examination (Table 2).

Table 2. Hatching percentage for cysts with different water content in first and second experiments

Variant	1st experiment		2d experiment	
	water content, %			
	1	68	3	68
Control	86,80±2,97	62,40±4,25	80,20±3,49	75,30±2,68
Flight	83,80±3,23	67,60±4,10	88,0±2,85*	81,60±2,40*

Table 3. Development (breaking shell, hatching nauplii) percentage among irradiated and nonirradiated, dry and moistened Artemia cysts (interior container)

Dose Gy	Variant	Moistened cysts			Dry cysts		
		cracking	hatching	nauplii	cracking	hatching	nauplii
0	Control	76,2±2,7	75,3±2,7	69,6±2,8	82,6±3,3	80,2±3,5	75,8±3,7
	Flight	85,5±2,4*	81,6±2,4*	72,6±2,8	88,8±2,8*	88,0±2,8*	82,2±3,3*
100	Control	67,5±2,9	66,8±2,9	61,2±3,0	85,4±3,1	81,6±3,2	75,6±3,8
	Flight	65,7±2,9	63,5±3,0	56,4±3,1*	82,0±3,4	82,0±3,4	70,0±3,0*
500	Control	61,7±3,0	60,5±3,0	55,2±3,1	82,4±3,3	80,0±3,5	50,6±3,4
	Flight	57,2±3,0*	56,7±3,1	49,7±3,1*	79,4±3,5	76,4±3,7	43,0±4,3*

Only one layer was examined in the study of irradiated cysts, the one with high development percentage. The results revealed that in pre-irradiated cysts the effect was more noticeable on the moistened cysts at all stages of development and on the dry ones only at the stage of floating larvae (nauplii). This effect was enhanced under spaceflight conditions (Table 3). As for the life span of irradiated and nonirradiated dry cysts, no differences were demonstrable between flight and control variants. The effects elicited by SFF on moistened cysts were manifested only in the case of a dosage of 500 Gy.

Exterior container. The results pertain only to dry cysts provided with minimum and maximum shielding. With minimum shielding, cysts exposed to doses of 3 to 16 cGy showed no changes at the stage of nauplius hatching, regardless of arrangement of layers, means of attachment or location of material in the stack. Conversely, with maximum shielding of cysts, the effects were a function of location of biolayers and arrangement of biological material. Thus, cysts arranged in a monolayer showed reliable decrease in hatching percentage regardless of level of development. In loosely arranged cysts, a decline in hatching was observed only in one case with use of a dosage of 0.12 cGy (Table 4).

The Bioblock-5 experiment revealed that the effect induced by SFF on Artemia cysts depends on factors related to the physiological condition of the biological material and how it was arranged (monolayer or loose). Postflight

retardation of development was found only in cysts in the interior container with low percentage of development. Let us recall that the Biostack [6, 7] experiments performed aboard Apollo 16 and 17 showed moderate capacity for development as manifested in cracking percentage (~60%).

Table 4. Percentage of hatching of cysts arranged in monolayer and loose outside the biosatellite

Cysts	Variant	Monolayer			Loose	
		dosage, cGy				
		0,12	3	15	0,12	15
High percentage of development	Control	84,60 ± 3,16	67,40 ± 4,11	80,60 ± 3,47	80,70 ± 3,65	81,0 ± 3,41
	Flight	73,40 ± 3,87*	69,00 ± 4,05	79,80 ± 3,52	75,2 ± 3,79*	82,2 ± 3,35
Low percent. of development	Control	50,20 ± 4,38	55,20 ± 4,36	45,40 ± 4,30	62,2 ± 4,25	61,6 ± 4,26
	Flight	40,60 ± 4,31*	50,60 ± 4,38	42,80 ± 4,34	65,5 ± 4,16	58,0 ± 4,33

The method of packing the biological material could also affect these parameters: inhibited development was demonstrated only for cysts arranged in a monolayer. The influence of means of stowing biological material was also demonstrated in earlier experiments--Bioblock-1 aboard Cosmos-782 [4], where inhibition of larval survival was manifested only in cysts packed in a monolayer.

Artemia cysts are characterized by a very low hydration percentage. Their water content can be artificially increased, but only in loosely packed cysts and in a sealed environment, unlike cysts packed in a monolayer. In the Bioblock-4 experiment performed aboard Cosmos-1129 [3], a slight decline in development was only observed in cysts with 50% hydration, whereas in the Bioblock-5 experiment the effect of water content was manifested only in cysts exposed to  $\gamma$ -rays before the flight.

Pre-irradiation enabled us to demonstrate the effect of SFF, since the decline in survival rate of larvae was more noticeable in flight group cysts. While this phenomenon was observed in cysts regardless of their hydration, viability, on the contrary, was different for dry and moistened cysts: the most appreciable decline in mean life span was observed after irradiating dry cysts. However, this effect did not occur in the flight group of cysts.

Studies pertaining to the first stage of development are indicative of increase in radioresistance of flight group material. These conclusions are consistent with the data obtained from the Bioblock-3 experiment [5], in which hatching constituted 39.6% for control cysts and 18.2% for those kept aboard the Salyut-7 station for 40 days, the dose of preflight irradiation of cysts being 1 Gy.

In the Bioblock-2 experiment, cysts packed loosely were used, and they were in direct contact with space. In spite of the fact that the flight lasted 20 days, there was no delay in development of cysts examined 2 and 4 months after the



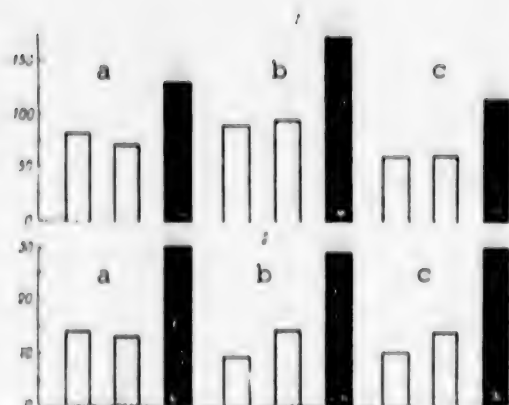


Figure 3.

Genetic effect and morphological abnormalities of tobacco seedling cotyledons

- I) number of changes/100 cotyledons  
 II) number of abnormal plants (%)  
 a, b) interior and exterior stacks, loose arrangement  
 c) monolayer in grid  
 White bars--laboratory control, hatched--transport control, black--flight

flight. In the Bioblock-5 experiment, cysts arranged loosely or in a monolayer received significantly different doses of cosmic radiation. In this case, the submitted results seem paradoxical or difficult to explain, since the effect of flight factors was manifested only in the container with maximum shielding, where absorbed dosage was 0.0012 Gy.

The cysts were shielded from ultraviolet radiation in the two exterior containers, but they were exposed to the effect of vacuum. Probably the demonstrated differences in reactions could be attributable to other causes, such as, for example, temperature. In view of the importance of studying the effect of cosmic radiation, it is desirable to continue with the investigations.

#### Studies of Tobacco (*Nicotiana tabacum*) Seeds

Figure 3 shows that the incidence of somatic reactions increases (2-fold) significantly in the cotyledons and first two leaflets in seeds of the flight

group, as compared to control group of seeds (laboratory and transport). It was also shown that there are no differences between groups of seeds packed loosely and in a monolayer, either within or without the biosatellite.

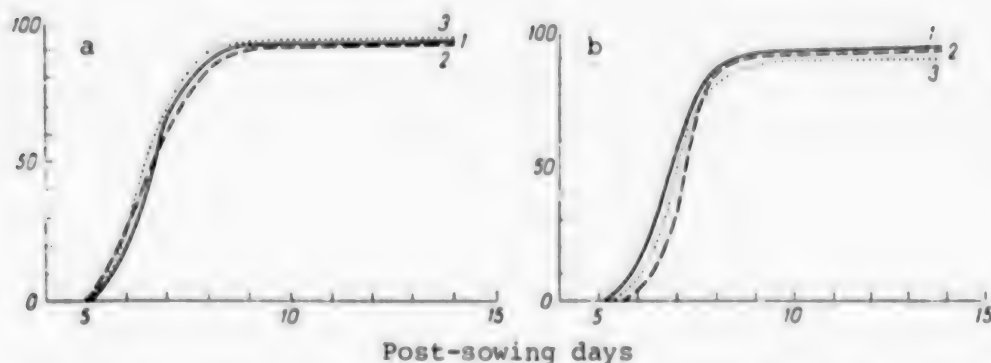


Figure 4. Dynamics of germination of tobacco seeds packed loosely (a) and in a monolayer (b)

X-axis, time after sowing (days); y-axis, number of germinated seeds at stage of 2 cotyledons (%)

- I) flight II) laboratory control III) transport control

As for other parameters, such as rate and percentage of germination (Figure 4), no reliable differences were found either between flight groups or between flight and control groups.



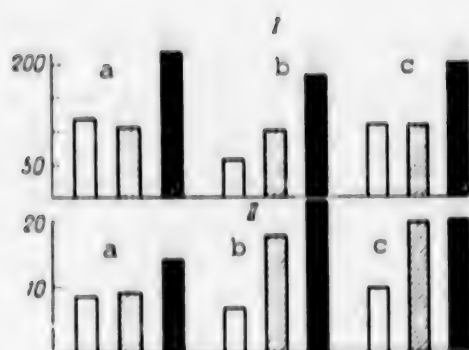


Figure 5.

Genetic effect and morphological anomalies on first two tobacco leaves

I) changes per 100 leaves

Other designations are the same as in Figure 3.

The incidence of morphological anomalies on seedlings and cotyledons (Figure 5) was rather high, and it was higher in the first two leaves of seedlings in the flight group than the transport control.

In the Bioblock-5 experiment, SFF elicited primarily change in the genetic system and an increase in incidence of morphological abnormalities of cotyledons. On the whole, we are dealing with new findings, if they are compared to preceding experiments where the same criteria were used: genetic, physiological and morphological in the Bioblock-2 experiment, genetic and physiological in the Bioblock-3 experiment.

These findings confirm the variability of effects elicited by the same SFF in different experiments in space.

Studies of isolated caryopses and embryos of rice (*Oryza sativa*). Investigation of the dynamics of 30-day germination of caryopses in flight and control variants failed to demonstrate reliable differences. After 15 days of cultivation, germination percentage was demonstrable, there was change in length of foliate stalks, roots. The differences found between flight and control variants were unreliable in all instances. Some of the caryopses were cut with a freeze microtome in order to perform an analysis using a Castaing microprobe. The purpose of the experiment was to demonstrate any possible differences in microdistribution of some minerals (potassium, magnesium, phosphorus, calcium and silicon), since this had been observed in specimens following long-term spaceflights. These studies failed to reveal reliable differences in distribution of minerals between flight and control caryopses.

The experiments performed within the framework of Bioblock-5 during a short-term flight failed to demonstrate reliable differences in the following criteria: microdistribution of mineral elements in caryopses, reaction of whole caryopses and embryos exposed to SFF, as compared to control specimens.

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INVESTIGATION OF GENETIC STRUCTURES OF RAT GERM CELLS FOLLOWING FLIGHT ABOARD  
COSMOS-1514 BIOSATELLITE DURING THEIR PRENATAL DEVELOPMENT

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21,  
No 6, Nov-Dec 87 (manuscript received 20 Nov 86) pp 24-27

[Article by D. K. Benova (Bulgarian People's Republic)]

[English abstract from source] Male rats that were flown on Cosmos-1514 during their prenatal days 13 through 18 were investigated. The animals were sacrificed when they reached sexual maturity. Preparations were made of their testes for cytogenetic analysis: spermatocytes were at the stages of diakinesis-metaphase I. The flown rats had 0.9% reciprocal translocations while the ground-based synchronous controls showed 0.5%. Exposure to space flight factors in combination had a mutagenic effect on gonocytes. However, the adverse effect of microgravity per se was not demonstrated unambiguously.

[Text] In the period of embryogenesis, man and mammals are highly sensitive to deleterious environmental factors. This sensitivity is specific for different tissues, and it depends on the stage of embryonic development.

Prior studies established that spaceflight factors have an effect on mutagenic activity of human and mammalian somatic cells [1, 6, 15]. However, such an effect was not established for germ cells of adult rats examined aboard Cosmos-936 biosatellite [2].

Our objective here was to determine the effect of spaceflight factors on the chromosome system of male rat germ cells during their embryonic development.

#### Methods

Wistar rats were aboard Cosmos-1514 from the 13th to 18th day of the gestation period. On the 23d day, flight rats bore offspring on the ground. We examined only males prior to puberty (they were sacrificed at the age of 102 days). Concurrently with the flight experiment, we set up a ground-based control with simulation on the ground of all flight conditions. A third group of rats at the same stage of the gestation period served as a vivarium

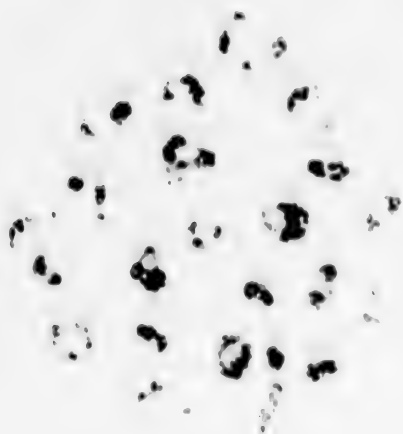


Figure 1.  
Normal spermatocyte plate in  
diakinesis-metaphase I

[14]. The 11 multivalents and several questionable metaphases detected in our experiments were analyzed by 3 other specialists. The data were submitted to statistical processing by means of nonparametric analysis with use of Fisher's criterion.

vivarium control. This group of animals was also sacrificed at the age of 101-102 days.

A preparation was made from the testes for cytogenetic analysis using a modification of the Dyban method [3, 10]. We analyzed approximately 200 spermatocytes in diakinesis-metaphase I (Figure 1). We took into consideration reciprocal translocations in the form of multivalent rings or chains (Figure 2). At this stage of spermatogenesis, 21 bivalents are formed in rats. Due to the presence of metacentric and submetacentric chromosomes in the karyotype, bivalents very often present a complicated and largely varying configuration. No doubt, this presents difficulties with respect to recording them, as compared to analysis of mice



Figure 2. Spermatocyte plate in diakinesis-metaphase I with  
translocation. Lens 100 $\times$ , eyepiece 12.5 $\times$ ; basic  
fuchsin dye

#### Results and Discussion

The results of the experiment are listed in the table. In flight animals, only 7 (0.9%) out of 815 analyzed cells had translocations. As compared to

the vivarium control, this difference was reliable ( $p < 0.01$ ). There was an increase in translocations in the ground-based control ( $p < 0.01$ ). Differences in yield of translocations between the group flown in space and its control on the ground were unreliable ( $p < 0.05$ ).

Reciprocal translocations in rats flown aboard Cosmos-1514 during period of embryonic development

Group		No of animal examined	Weight, g	Number of cells analyzed	Cells with trans- locations %	Type (and number) of associations	ring	chain
Spaceflight	4	1	375	209	0.5	—	1	
		5	375	198	0.5	1	—	
		9	392	208	1.5	1	2	
		12	413	200	1.0	1	1	
		Mean 388.7	Total 815	Mean 0.9	Total 3	4		
Ground- based control (flight simulation)	4	55	280	200	1.5	1	2	
		59	326	198	0.0	—	—	
		70	350	199	0.5	1	—	
		78	408	201	0.0	—	—	
		Mean 341	Total 798	Mean 0.5	Tot. 2	2		
Vivarium control	4	27	375	200	—	—	—	
		34	385	200	—	—	—	
		48	390	200	—	—	—	
		49	392	203	—	—	—	
		Mean 385.5	Tot. 803	—	—	—		

Germ cells of rats on the 13th-18th day of the gestation period are primordial germ cells, i.e., gonocytes. They are notable for high sensitivity to ionizing radiation [14]. Mice have shown significantly higher radiosensitivity of gonocytes (peak sensitivity is demonstrable on the 16th day after fertilization) [4]. In rats, their differentiation in  $T_1$  spermatogonia starts on the 18th day [11]. However, most researchers observe that genetic radiosensitivity of gonocytes according to parameter of reciprocal translocations does not differ from that of adult specimens [7, 9, 12].

The reciprocal translocations in rat spermatocytes indicate that space factors induce mutations in gonocytes, though to an insignificant extent. These mutations are apparently attributable not only to weightlessness, but other extreme flight conditions [1]: vibration, accelerations, noise, impact accelerations [8]. However, we cannot totally rule out the influence of weightlessness, since the level of induced translocations is low, and the number of animals examined is insignificant.

The studies of Vglenov et al. [2] failed to demonstrate a mutagenic effect of a 1-day spaceflight on germ cells of adult rats. This can be attributed, to



some extent, to the significant sensitivity of spermatogenesis at these stages (chiefly spermatogonia B and stage of intermediate type). Most of the cells with translocations have probably already perished. The adverse effect of space factors on mouse germ cells had been established in the experiments of R. P. Kusheva and I. A. Bayev [5].

In this investigation, we studied the effect of space factors on gonocytes, which are more sensitive. However, they, like stem spermatogonia of adult specimens, most probably constitute a population that is heterogeneous in sensitivity. As long as the gonocytes are at the spermatocyte stage, at which they are analyzed, they undergo numerous stages of cellular differentiation. The more sensitive gonocytes had apparently perished. However, the gonocytes originating from this population with lower sensitivity retain the genetic lesion and reach the spermatocyte stage.

Chromosome translocations offer virtually no hindrance to cell division. Apparently, these cells can retain the genetic lesion caused by space factors.

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MORPHOLOGICAL AND FUNCTIONAL STATE OF THE HYPOTHALAMO-HYPOPHYSEAL NEUROSECRETORY SYSTEM OF RATS FLOWN ABOARD COSMOS-1667

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (manuscript received 11 Mar 87) pp 27-31

[Article by Ye. I. Alekseyev]

[English abstract from source] Morphological and morphometric data were used for comparative analysis of changes in the major compartments of the hypothalamic-pituitary neurosecretory system of Cosmos-1667 experimental and control rats. The flown rats showed morphological signs of inhibited ADH production. This inhibition may facilitate fluid excretion and development of a new hydration level at an early stage of adaptation to micro-gravity. The enhanced ADH secretion from neurons of supra-optic nuclei of the hypothalamus and axons of the posterior pituitary may lead to water retention and compensation of water loss after return to Earth's gravity.

[Text] The hypothalamo-hypophyseal neurosecretory system (HHNS) is, as we know, the main producer of antidiuretic hormone (ADH)--vasopressin, which regulates fluid balance and tonus of small vessels [1, 4, 10]. In experiments aboard biosatellites of the Cosmos series, it was shown that returning animals (rats) to earth's gravity following long-term (18-22-day) flights activates HHNS function, which increases progressively for the first 2 days of observation [6, 7].

We submit here the results of morphological, histochemical and karyometric studies of neurons of the supraoptic nuclei of the hypothalamus, as well as neurosecretory axons and pituicytes of the posterior lobe of the rat hypophysis following a short-term (7-day) flight aboard Cosmos-1667.

#### Methods

The hypothalamus and hypophysis of 4 male Wistar-SPF rats (weight 300 g, age 3 months) decapitated 4-6 h after landing of the biosatellite, 4 animals from the ground-based control experiment (GCE) and 5 animals from the vivarium control (VC) served as material for our investigation. In addition, we used the results of examining the hypothalamus and hypophysis of rats used in additional experiments with 4- and 8-h rotation on a centrifuge at 2 G

(simulation of gravity stress) and 7-day clinostatic hypokinesia, as one of the physiological models of weightlessness, in order to make a differential determination of changes arising during and after flight. In each group of additional experiments, we used 5 male Wistar-SPF rats (age 3 months, sacrificed by decapitation). In all of the experiments, glandular tissue was fixed in Bouin fluid, imbedded in paraffin and series of horizontal sections 5  $\mu$ m in thickness were prepared. Identification of neurosecretory substance (NSS) in neurons of supraoptical nuclei (SON) of the hypothalamus and axons of the posterior lobe of the hypophysis was made by staining the sections with fuchsin paraldehyde after Gomori-Gabu, while demonstration of ribonucleoproteins (RNP) in neurosecretory cells was made with galloxyanin after Einarson. Karyometry was performed using an RA-6 drafting instrument to draw 100 nuclei of neurons of SON and neurohypophyseal pituicytes from each rat at linear magnification of 3000 $\times$ . Subsequent measurement of diameters of nuclei, computation of their volume and statistical processing of quantitative data were performed using the conventional methods of morphometry.

## Results and Discussion

As a rule, two basic groups or subpopulations of neurosecretory cells are demonstrable in the rat hypothalamus SON, which differ in dimensions, shape and staining of their cytoplasm with dyes. These are the so-called "light" neurons, which are large, oval-spherical, and "dark" cells, which are small and of an irregular shape. It is believed that the light neurons effect synthesis and secretion of ADH-vasopressin, whereas the dark ones are a spare pool of cells [3-5]. In male rats, neurons of the light type prevail quantitatively over the dark ones, and they determine in essence the functional state of SON. For this reason, in our study we concentrated mainly on evaluation of the functional distinctions of subpopulations of light neurons.

In the flight group (FG) of rats, SON presented a morphological structure that is similar to that of VC and GCE animals: light and dark neurons retained their inherent dimensions and shape, and most of the cells were variants of light ones. However, functionally, the light type of neurons differed considerably from those of VC and GCE animals. When hypothalamus preparations were stained with galloxyanin and fuchsin paraldehyde, all FG rats showed very marked reduction in total ribonucleoprotein and neurosecretory substance content in the cytoplasm of light cells. Unlike the control, RNP substance had low light-optical density and acquired the appearance of barely discernible narrow rims or fine, friable clumps concentrated in the vicinity of the cell membrane (see Figure, *a* and *z*). In most neurons there were virtually no accumulations of NSS in the perinuclear zones of synthesis, which are usually inherent in normal cells [8, 9], and isolated granules were demonstrable only in a few cells around the nuclei (see Figure, *c* and *d*). The decrease in RNP and NSS content in neuronal cytoplasm was associated with reliable decrease in volume of their nuclei. In GCE rats, the bodies of SON neurons could virtually not be distinguished from those of VC animals, with respect to RNP and NSS content; however, the volume of their nuclei was enlarged (see Table). Unlike VC and GCE rats, those in the FG showed a large number of dilated axons on the level of the SON and medial eminence. No NSS was demonstrable in the lumen of many of them. At the same time, there was distinct decline of NSS optical density in the posterior pituitary

lobe, in both most fine dilatations (terminals) of axons and in wider ones--Herring bodies (see Figure, *a* and *e*). The decrease in NSS content of the neurohypophysis of flight animals was associated with distinct hypertrophy of pituicytes which, as we know, are involved in processes of excretion of neurohormones into the vascular stream [5, 11]. On sections of the gland, there was prevalence of larger pituicytes than in VC and GCE animals, with rather pronounced process-containing outlines. The nuclei of such cells were somewhat smaller than usual (see Table).

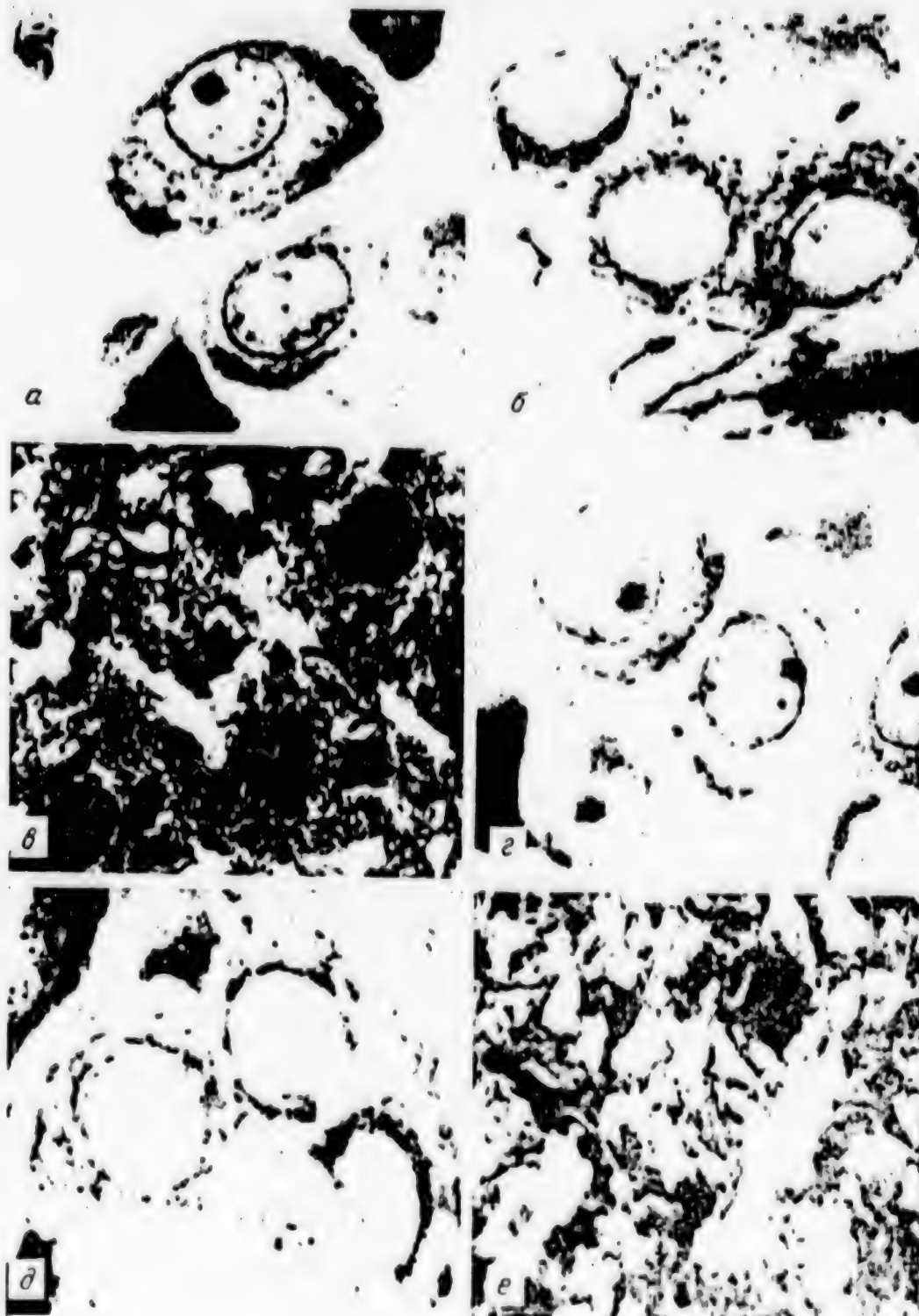
Results of karyometry of neurons of hypothalamic SON and pituicytes of posterior lobe of the rat hypophysis ( $M \pm m$ )

Animal group	Volume, $\mu m^3$			
	neurons	<i>p</i>	pituicytes	<i>p</i>
VC	402,0 $\pm$ 10,2		132,2 $\pm$ 2,3	
FG, 7 days	341,9 $\pm$ 6,3	<0,001	121,2 $\pm$ 2,0	<0,02
GCE	464,8 $\pm$ 6,9	<0,001	130,0 $\pm$ 3,0	<0,6
Rotation on centrifuge:				
4 h	416,3 $\pm$ 18,9	<0,6	126,1 $\pm$ 3,2	<0,2
8 h	417,0 $\pm$ 8,5	<0,6	128,5 $\pm$ 2,8	<0,2
Hypokinesia (7 days)	404,8 $\pm$ 8,1	<0,6	135,0 $\pm$ 3,5	<0,6

Note: Parameter *p* is given in comparison to the control.

On the whole, the study of FG rat HHNS enabled us to demonstrate two main types of changes: signs of intensification of NSS transport from the bodies of SON neurons in axons given off from them and signs of diminished activity (level) of ADH synthesis, which was manifested by reduction in size of neuronal nuclei and virtually total absence of NSS granules in the zones of its synthesis, as well as distinct decrease in overall pool of NSS on the level of the posterior lobe of the hypophysis. To interpret these changes, we used the results obtained from studies of analogous elements of the rat's neurosecretory system from experiments with rotation for 4 and 8 h on the centrifuge, which simulated hypergravity when the animals returned from weightlessness to earth's gravity (acute gravity stress). It was established that 4-h rotation on the centrifuge elicits intensive discharge (excretion) of neurosecretory substance from SON neurons, which is associated with dilatation of most axons that was just as marked as in FG rats. The lumen of the latter contained considerably less granular secretions than in VC and GCE animals. Discharge of NSS from SON cells and their axons led to significant accumulation of hormonal substance, chiefly in the fine dilatations of neurosecretory fibers on the level of the posterior lobe of the hypophysis, whereas Herring bodies retained their normal dimensions and density of NSS deposited in them. Upon termination of 8-h rotation on the centrifuge, there was normalization of NSS content, not only in neurons and axons branching from them, but over all the fine dilatations of neurosecretory fibers of the posterior lobe of the pituitary. It should be noted that 4- and 8-h exposure to hypergravity did not lead to change in intensity of ADH synthesis in SON: the usual accumulations of neurosecretory granules were demonstrable around the neuronal nuclei. In both experiments, total RNP content of cell cytoplasm remained comparable to the control.





#### Rat hypothalamus and posterior lobe of hypophysis

- a, c, e*) control; distinct consolidations of RNP (*a*) on cytoplasm periphery and accumulations of NSS granules (*c*) near nuclei of SON neurons; lumen of small and large axons moderately filled with neurosecretions on level of posterior lobe (*e*)
- b, d, f*) experiment (4-6 h after flight); dramatic decrease in RNP (*b*) and NSS (*d*) content in SON neurons; neurosecretion content is noticeably reduced in all variants of posterior lobe (*f*) axons
- Lens 100 $\times$ , eyepiece 7 $\times$  (*a, b, c, d*); lens 40 $\times$ , eyepiece 7 $\times$  (*e, f*)
- Stain: gallocyanin (*a, b*) and fuchsin paraldehyde (*c, d, e, f*)

Examination of the hypothalamus and hypophysis after submitting rats to 7-day clinostatic hypokinesia failed to reveal morphologically significant functional changes in the principal elements of HHNS. In particular, RNP and NSS content of SON neurons, as well as NSS content of axons in the posterior lobe of the hypophysis, was the same as in VC rats. Seven-day restriction of activity, like 4- and 8-h simulation of hypergravity, was not associated with changes in volume of SON neuronal nuclei or pituicytes of the neurohypophysis (see Table).

Thus, a comparison of the above facts shows that no morphological signs of depressed production of ADH-vasopressin in SON neurons was demonstrable in any of the ground-based model experiments. Intensification of mobilization of neurosecretions from SON neurons and endings of their axons on the level of the posterior lobe of the hypophysis, similar to that of FG rats, was demonstrated only in the experiment with 4-h rotation on the centrifuge. On this basis, it can be assumed that the decrease in intensity of synthesis (production) of ADH occurred in weightlessness, while the signs of its accelerated mobilization from all elements of the HHNS developed upon termination of the flight under the effect of acute gravity stress [2, 7, 12].

Inhibition of ADH-vasopressin production could have been instrumental in discharge of fluid and establishment of a new hydration level at the first stage of adaptation to weightlessness, whereas intensification of hormone secretion into blood in the early postflight hours is apparently involved in fluid retention and compensation of its loss related to the rats' return to earth's gravity.

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INVESTIGATION OF RAT SKELETAL MUSCLES FOLLOWING SHORT-TERM SPACEFLIGHT  
ABOARD COSMOS-1667 BIOSATELLITE

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21,  
No 6, Nov-Dec 87 (manuscript received 12 Sep 86) pp 31-35

[Article by Ye. I. Ilyina-Kakuyeva]

[English abstract from source] Using morphological and histochemical methods, skeletal muscles (soleus, gastrocnemius, quadriceps and biceps muscles) of Wistar-SPF rats flown for 7 days on Cosmos-1667 were investigated. The short-term exposure to microgravity led to muscle atrophy which primarily involved myofibers with a high level of oxidative metabolism and a low level of ATPase activity. The percentage composition of myofibers of different types remained unchanged. The soleus muscle showed the greatest changes which included both atrophic and dystrophic shifts. Muscle atrophy developed together with metabolic changes that resulted in glycogen accumulation and decreased SDH activity. After return to Earth's gravity microcirculation disorders were seen only in the soleus muscle.

[Text] Data obtained from a morphological and histochemical study of skeletal muscles of rats flown aboard biosatellites of the Cosmos series for 18.5 to 22 days revealed that atrophy develops in muscle tissue under the effect of weightlessness, its severity varies in different muscles, and there is alteration of metabolism manifested chiefly by accumulation of glycogen and lipids in muscle fibers [1]. We conducted this study in order to determine how soon after the start of a flight the atrophic process and metabolic changes appear in muscles. In addition, our objective included investigation of the muscular microcirculatory system, since there is extremely sparse information concerning its state following spaceflights [4].

#### Methods

We examined the following muscles of 7 male Wistar rats of the SPF colony exposed for 7 days to weightlessness aboard Cosmos-1667, as well as 7 rats in the vivarium control group: soleus, gastrocnemius (lateral portion), femoral quadriceps (central portion) and brachial biceps. The animals were sacrificed by decapitation 4-8 h after landing. The muscles were weighed,



fixed in 10% neutral formalin prepared on a phosphate buffer and imbedded in histoplast [plastic]. For histochemical analysis, pieces of muscle were frozen in freon-12 cooled with liquid nitrogen and cut in a cryostat. To study the microcirculatory system in cross sections of muscles imbedded in histoplast, we demonstrated functional capillaries, staining their red cells with Heidenhain's iron hematoxylin and counting their number per 200 muscle fibers.

Histochemical examination included a comparative study of amounts of glycogen in muscle fibers (PAS reaction), phospholipids (sudan black B stain), activity of phosphorylase [13], succinate dehydrogenase and  $\alpha$ -glycerophosphate dehydrogenase (GPDH) unbound with NAD [12]. Substrate levels and enzyme activity were assessed visually. Myosin ATPase activity was determined by the method of H. A. Padykula and E. Herman [11], with prior fixing for 5 min in 10% neutral formalin solution containing 2%  $\text{CaCl}_2$  (pH 9.2) at 4°C temperature, for identification of different types of muscle fibers, their morphometry and determination of percentile amounts in cross sections of muscles.

According to one of the classifications that served as our basis, muscle fibers were identified on the basis of their size and level of activity of oxidative enzymes, making a distinction between three types: type I--red, small, with high enzymatic activity; type II--white, large, with low enzymatic activity; type III--intermediate, with moderate enzymatic activity. It was established that preincubation in Ca-formol permits immediate demonstration in sections of phasic mixed muscles of six types of fibers. Thus, among the small red fibers we could single out two independent types, one of which has extremely high ATPase activity and the other has virtually none. We named these fibers red A and B, respectively. White large muscle fibers have moderate ATPase activity which, depending on which motor unit they belong to, may vary somewhat. In spite of the fact that intermediate fibers have the same, above-average enzyme activity, we also divided them into two types on the basis of their size, designating the larger ones as type A and the smaller ones, type B (Figure 1a). The sixth type of muscle fiber is large, wanting in ATPase activity and was encountered only in the quadriceps. Two types of fibers are demonstrable in the tonic soleus: with high and low ATPase activity (Figure 1b).

The cross section area (CSA) of muscle fibers was determined by gravimetry [3]. All of the data were submitted to statistical processing by the Student method. Sections of frozen muscles were stained with hematoxylin and eosin, as well as after Mallory, for general histological examination.

## Results and Discussion

Rat weight did not change during the flight, constituting  $332 \pm 4.4$  g in the experiment and  $333 \pm 6.8$  g in the control. Muscular response to 7-day weightlessness varied. The soleus was affected the most and its weight diminished by 22.7%, whereas the decrease in weight of the gastrocnemiums and brachial biceps constituted 10.7 and 12.3%, respectively, that of the quadriceps remaining unchanged. Morphometric examination revealed that onset of an atrophic process was one of the causes of reduction in muscle mass. In white muscles, only one type of fiber was subject to atrophic change, the small ones, without ATPase activity and with high level of oxidative metabolism

(red B). Thus, fiber CSA decreased by 20 and 15.7%, as compared to the control in the gastrocnemius and brachial biceps, respectively. In the tonic soleus, CSA decreased in both types of fibers, those with high oxidative metabolism and high ATPase activity (by 14.4%) and those with moderate oxidative metabolism and low ATPase activity (by 17.6%). CSA of quadriceps fibers in experimental animals did not differ from the control (Table 1).



Figure 1. Myosin ATPase activity in different types of fibers  
a) gastrocnemius; fiber types: 1) white, 2) red A, 3) red B, 4) intermediate A, 5) intermediate B  
b) soleus; fiber types: 1) red A, 2) intermediate

Table 1.  
Area of muscle fiber cross section ( $M^2m$ )

Muscle	Fiber type	Vivar. control	Flight
Soleus	Red	$3.18 \pm 0.23$	$2.98 \pm 0.09^*$
	Intermediate	$1.27 \pm 0.11$	$3.52 \pm 0.11^*$
Gastrocnemius	White	$3.06 \pm 0.29$	$1.59 \pm 0.21$
	Red A	$2.22 \pm 0.15$	$1.98 \pm 0.09$
	" B	$2.56 \pm 0.13$	$2.05 \pm 0.11^*$
	Intermediate A	$3.18 \pm 0.11$	$1.01 \pm 0.09$
	Intermediate B	$2.18 \pm 0.03$	$2.26 \pm 0.01$
Femoral quadriceps	White	$6.18 \pm 0.32$	$6.57 \pm 0.05$
	Red A	$1.69 \pm 0.08$	$1.61 \pm 0.12$
	" B	$1.83 \pm 0.06$	$1.78 \pm 0.16$
	Intermediate A	$1.15 \pm 0.09$	$1.26 \pm 0.28$
	Intermediate B	$2.29 \pm 0.04$	$2.49 \pm 0.03$
Brachial	White	$1.96 \pm 0.18$	$1.58 \pm 0.17$
	Red A	$1.12 \pm 0.03$	$1.31 \pm 0.03$
	" B	$1.41 \pm 0.03$	$1.19 \pm 0.07^*$
	Intermediate A	$2.13 \pm 0.05$	$2.29 \pm 0.06$
	Intermediate B	$2.07 \pm 0.02$	$2.06 \pm 0.01$

\*Statistically reliable differences between experiment and control.

While the changes in the gastrocnemius and brachial biceps were limited in essence to a slight atrophic process, there were signs of dystrophy in the soleus, in addition to atrophy. They were manifested by appearance of fibers with uneven distribution of reaction product--formazan--in demonstration of oxidative enzymes, individual target fibers, target-like and triangular fibers (Figure 2c). In one of the rats, we observed in this muscle local dilatation of endomysium layers and proliferation of connective tissue cells around vessels (Figure 2d and e). Estimation of percentage of different types of fibers in muscles failed to reveal differences between the experiment and control.

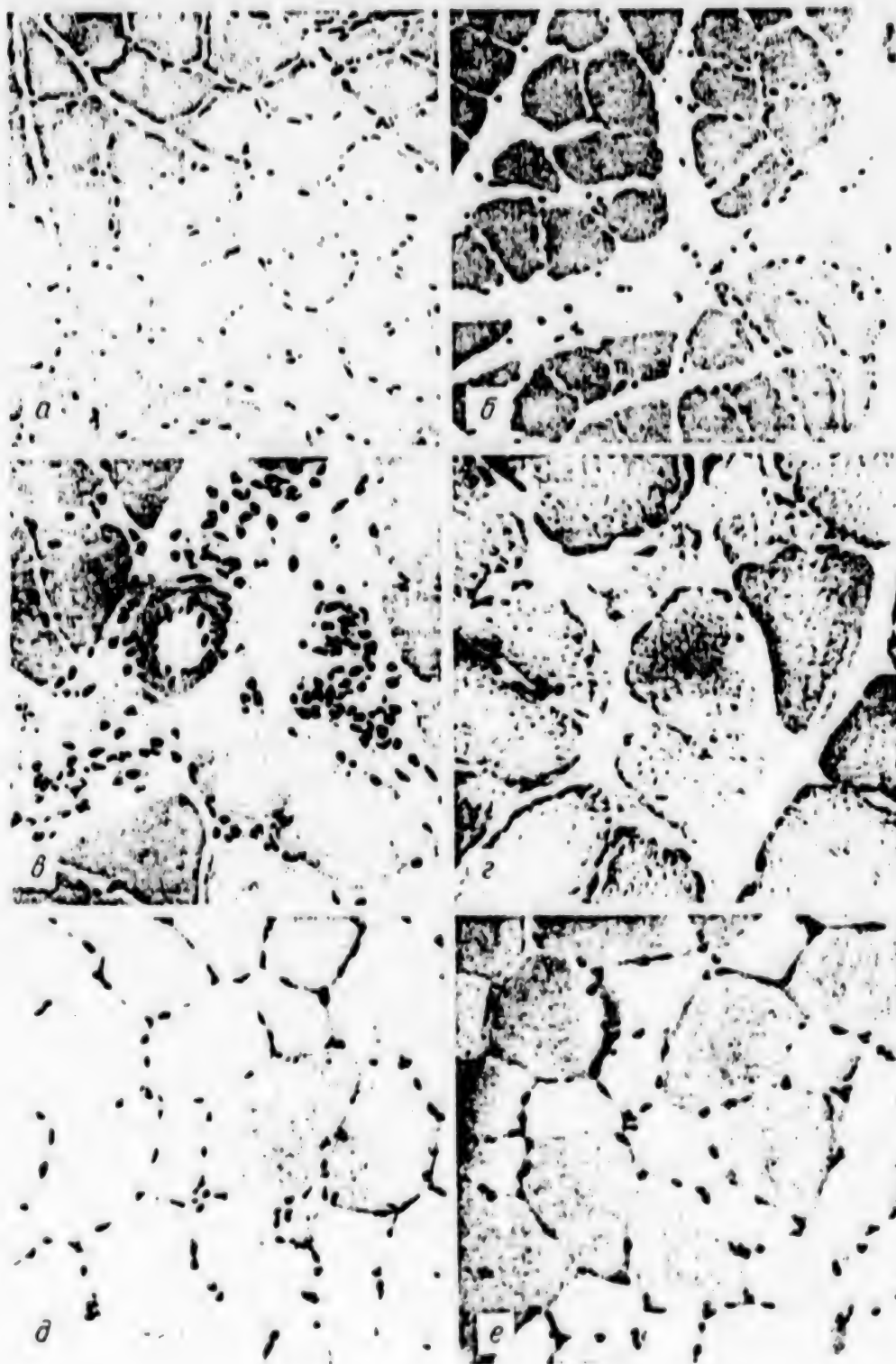


Figure 2. Soleus

- a) control
- b) dilated layers of endomysium indicative of edema in muscle; lens 10×, eyepiece 10×
- c) proliferation of connective tissue cells around vessels; hematoxylin and eosin stain

[Caption continued on page 47]

Caption for Figure 2, continued:

2) target fiber surrounded by atrophic fibers with uneven distribution of diformazan upon demonstration of SDH

δ) glycogen content of control animal gastrocnemius

ε) same for experimental animal

PAS reaction; lens 16×, eyepiece 10×

Table 2.  
Number of functional capillaries per  
200 muscle fibers ( $M \pm m$ )

Muscle	Vivarium control	Flight
Soleus	156,3 ± 10,45	149,5 ± 17,72
Gastrocnemius	119,5 ± 5,76	113,4 ± 7,89
Femoral quadriceps	133,5 ± 10,10	130,1 ± 20,70
Brachial biceps	115,4 ± 5,21	109,5 ± 11,70

We were impressed by the higher glycogen content in all muscles of experimental animals, as compared to the control (Figure 2δ and ε). Glycogen was demonstrable even in small fibrils with high oxidative metabolism, which are usually wanting in this substrate. The increase in glycogen content was associated with increase in phosphorylase activity. All muscles showed a decrease in succinate dehydrogenase (SDH) activity, and GPDH activity increased significantly only in the soleus.

A count of functional capillaries in all four muscles failed to reveal differences between experiment and control (Table 2).

Thus, this investigation revealed that an atrophic process arises in muscles at the early stages of a flight. In twitch muscles, this applied primarily to fibers wanting in ATPase activity with high oxidative metabolism. In tonic muscles, both types of fibers are affected, those with both high activity of oxidative enzymes and ATPase, and those with moderate activity of oxidative enzymes and low ATPase activity. With extension of flight duration, as indicated by data obtained in experiments aboard Cosmos-936, other types of fibers are involved in the atrophic process of white muscle, while the degree of atrophy of both types of fibers in the red soleus increases [1].

There are data in the literature to the effect that the pattern of muscle fibers can change when one type of fiber is transformed into the other under the effect of some factor or other, more often related to impairment of innervation processes [6, 8, 10]. Retention of the normal percentage of different types of fibers in this experiment serves as indirect evidence of absence of appreciable changes in the myoneural system as a whole, and only the appearance of isolated target fibers and target-like fibers in the soleus is indicative of the possibility of some local changes in innervation of this muscle [9].

Histochemical investigation revealed that there is increase in glycogen content of muscle tissue, which is associated with increase in phosphorylase activity. Accumulation of glycogen, but to a greater extent, was also noted in the muscles of animals flown in space for 18.5 days [1]. In this regard, the hypothesis was expounded that there are changes in carbohydrate metabolism of muscles during flight, which lead to accumulation of substrate, and that



this process arises at different stages of flight, progressing as its duration increases. At the present time, information has appeared in the literature to the effect that stress due to rotation of animals on a centrifuge may be the cause of accumulation of glycogen [7]. Considering that animals develop postflight stress in response to returning to earth's gravity and the accelerations associated with touchdown, we could not reject the hypothesis that expressly stress is the cause of accumulation of glycogen observed in muscles.

In favor of the first hypothesis is the fact that accumulation of glycogen in the experiment with a centrifuge was demonstrated only upon rotation for 24 h, and it was not found with 4-h exposure to accelerations. In addition, in a special experiment with 6-h rotation of rats on a centrifuge at 2 G, we also failed to demonstrate an increase in glycogen content of muscles. The animals had been dissected 4-8 h after flight.

In earlier flight and model experiments, along with structural damage to muscle fibers, there was significant increase in their GPDH activity. The hypothesis was expounded that the need to utilize lipids released upon dissociation of membrane structures of muscle fibers subject to atrophic and dystrophic processes is the cause of increase in activity of this enzyme [3]. Evidently, the same can explain the increase in GPDH activity of the soleus in our experiment. The cause of decline in SDH activity of muscle tissue, i.e., in the enzyme of the Krebs' cycle, in both this experiment and during long-term flights, remains unclear. Perhaps, the decline in SDH activity reflects a decline in general oxidative potential of muscles [5].

A count of functional capillaries in muscles revealed that no appreciable alteration of the microcirculatory system occurs in a short-term flight. Nevertheless, it is known that in the case of long-term flights, some time after landing edema develops in the soleus, which is indicative of diminished vascular tonus in this muscle [2]. Development of edema in the soleus of one of the rats in this experiment indicates that vascular tonus in this muscle decreases already at the early stage of exposure to weightlessness.

Thus, our investigation revealed that the process of muscular atrophy develops in rats already in the acute period of adaptation to weightlessness: in twitch muscles, red fibers with high oxidative metabolism and low ATPase activity are the first to be affected. The atrophic process in the soleus is associated with dystrophic changes. Signs of atrophy are combined with alteration of muscles manifested, in particular, by accumulation of glycogen in muscle fibers.

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MORPHOLOGICAL STUDY OF EARLY CHANGES IN RAT BONES IN SIMULATED WEIGHTLESSNESS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (manuscript received 31 Jan 86) pp 36-39

[Article by A. S. Kaplanskiy, Z. F. Sakharova, Ye. I. Ilyina-Kakuyeva and G. N. Durnova]

[English abstract from source] By histomorphometric methods tibial bones and lumbar vertebrae of rats exposed for 7 days to hypokinesia or head-down suspension were investigated. Both hypokinesia and suspension led to osteoporosis of the tibial metaphyseal spongiosa which was primarily induced by bone growth inhibition and probably by increased bone resorption. No signs of osteoporosis were seen in tibial diaphyses. In contrast to tibial bones, osteoporosis of the spongiosa of lumbar vertebrae was found only in hypokinetic rats. It is concluded that during exposure to simulated microgravity early signs of osteoporosis occur in the tibial spongiosa and that changes in the spongy matter of tubular bones and vertebrae are similar and systemic. It is suggested that an acute stress-reaction in response to simulated microgravity plays a certain role in the development of osteoporosis.

[Text] Experiments pursued aboard biological earth satellites of the Cosmos series revealed that 18-22-day exposure of rats to weightlessness leads to inhibition of bone growth, development of osteoporosis and decrease in bone strength [4-8]. Analogous or similar changes were also observed in rat bones with simulation of weightlessness by means of restricting movements of animals [3] or by "suspending" them by the tail in antiorthostatic position [10, 11]. We have made an effort here to determine how fast the above changes appear and the nature of early morphological signs of osteoporosis in experiments on rats submitted to hypokinesia or suspension.

#### Methods

The tibia and lumbar vertebrae from 38 male Wistar rats, with base weight of about 180 g, served as the material for our study. Experimental groups of rats were kept in tight box-cages for 7 days ("hypokinesia" group, 10 rats) or else suspended by the tail in antiorthostatic position by the method of Ye. A. Ilyin and V. Ye. Novikov [2] in such a way that they touched the

floor with their forelegs and could move about the cage ("suspension" group, 8 rats). As a control, we used nine rats from the same batch (vivarium control). In addition, prior to the experiment we sacrificed 10 intact rats which made up the baseline control group. Upon termination of the experiment, the rats were weighed and sacrificed by decapitation on a guillotine. The muscles were removed from tibiae and lumbar vertebrae, which were then fixed in 4% neutral formalin prepared with 10% EDTA solution. After 2 days, the bones were transferred to 10% EDTA for decalcification, which took about 10 days. After decalcification, the proximal third of the tibia was separated, and a segment about 2 mm tall was excised from the diaphysis at the level of attachment of the fibula. The proximal part of the tibia, segment of diaphysis and lumbar vertebrae were washed in tap water, dehydrated in alcohols and imbedded in histoplast [plastic]. Longitudinal serial sections of the proximal part of the tibia and lumbar vertebrae cut parallel to the frontal plane and transverse sections of the tibial diaphysis were stained with hematoxylin and eosin, picrofuchsin, methyl green-pyronine, alcian blue, toluidine blue and by the Schmorl method. An MOV-15 ocular micrometer was used to measure thickness of the epiphyseal cartilaginous growth plate and width of the zone of primary spongiosa on sections of the proximal tibia and lumbar vertebrae. Volume density of primary and secondary spongiosa of tibiae and lumbar vertebrae was determined by the point method, using the morphometric grid of S. B. Stefanov. The latter was also used to count haversian canals in compact matter of tibial diaphyses. The number of osteoblasts and osteoclasts in the zone of primary spongiosa of lumbar vertebrae and tibial metaphyses was counted in 20 and 50-70 fields, respectively, at 630 $\times$  magnification. Area of compact bone, bone marrow canal, osteocytic lacunae and haversian canals in tibial diaphyses were determined by a morphogravimetric method, and for this purpose we drew the outlines of the above structures on standard paper using an RA-6 drafting instrument, then cut out the patterns and weighed them, obtaining data expressed in arbitrary units. The obtained digital data were submitted to statistical processing by the Student method, considering differences to be reliable at  $p < 0.05$ .

## Results and Discussion

Morphometric studies of the proximal segments of tibial bones revealed that the width of the epiphyseal cartilaginous growth plate and zone of primary spongiosa, as well as volume density of primary and secondary spongiosa, number of osteoblasts and osteoclasts in the zone of primary spongiosa of rats in the vivarium control did not differ from the baseline control group. At the same time, there was statistically reliable narrowing of the cartilaginous growth plate and zone of primary spongiosa in rats submitted for 7 days to hypokinesia or suspension. There was also a decrease in volume density of primary and secondary spongiosa and increase in number of osteoclasts due mainly to appearance of mononuclear and binuclear forms (Table 1). The number of osteoblasts did not change in both experimental groups of rats, but there were qualitative changes in the osteoblast population. Thus, while there was prevalence of large, polygonal osteoblasts with extensive, markedly pyroninophilic cytoplasm and large "cavity" in the perinuclear zone in control groups of rats, in animals submitted to hypokinesia or suspension there was prevalence of smaller, spindle-shaped and polygonal cells. The

latter often presented vague outlines and a relatively small vacuolized cytoplasm without "cavity," i.e., there was prevalence of moderately active and minimally active cells [13], or cells with signs of dystrophic lesions.

Table 1. Results of morphometric studies of growth plate and spongiosa of proximal tibial metaphyses and lumbar vertebrae of rats (M±m)

Animal group	Growth plate width, $\mu\text{m}$	Primary spongiosa width, $\mu\text{m}$	Primary spongiosa vol.den-sity, %	Secondary spongiosa vol.den-sity, %	Osteo-blasts per field	Osteo-clasts per field
Tibial metaphyses						
Baseline control	427±26	543±44	47.3±1.0	22.5±1.0	40.0±2.0	1.54±0.10
Vivarium control	451±53	580±55	47.0±0.8	25.1±2.0	36.0±1.5	1.80±0.10
Hypokinesia (7 days)	304±13*	331±29*	35.9±1.4*	16.4±1.5*	39.7±1.8	2.41±0.11*
Suspension (7 days)	301±7*	334±15*	36.0±1.0*	15.9±1.6*	39.8±2.4	2.12±0.07*
Lumbar vertebrae						
Baseline control	136±3	180±10	41.4±1.7	22.7±1.2	16.0±1.1	2.3±0.1
Vivarium control	131±3	170±9	39.0±1.8	22.6±1.0	16.5±1.1	2.8±0.2**
Hypokinesia (7 days)	110±4*	110±5*	24.0±1.6*	18.2±1.3*	12.2±1.3*	2.7±0.2
Suspension (7 days)	120±4*	110±9*	33.6±2.5	20.0±1.2	13.1±1.2	2.4±0.2

\*Statistically reliable ( $p<0.05$ ) differences between experiment and vivarium control.

\*\*Statistically reliable differences between vivarium and baseline control.

Table 2. Results of morphometric analysis of rat tibial diaphyses (M±m)

Animal group	Compact subst. area, AU	Medullary cavity area, AU	Number of haversian canals	Haversian canal area, AU	Osteocyt. lacunae, area, AU
Baseline control	0.42±0.023	0.41±0.025	125.9±10.6	0.035±0.001	0.019±0.002
Vivarium control	0.41±0.023	0.52±0.067	137.1±12.3	0.037±0.002	0.021±0.001
Hypokinesia (7 days)	0.41±0.016	0.54±0.037	146.0±11.9	0.039±0.003	0.021±0.001
Suspension (7 days)	0.43±0.031	0.43±0.052	163.5±11.5	0.038±0.002	0.023±0.001

\*Statistically reliable ( $p<0.05$ ) differences between experiment and vivarium control.

Examination of tibial diaphyses revealed that there was no change in area of compact substance, bone marrow cavity or osteocytic lacunae, as well as in number of haversian canals and their area in both experimental groups of rats (Table 2). Morphometric analysis of spongiosa of lumbar vertebrae of control groups of rats failed to establish reliable differences in parameters measured, with the exception of number of osteoclasts, which was somewhat higher in the vivarium control than in the baseline control group. A decrease in width of the growth plate and primary spongiosa zone was observed in vertebrae of both experimental groups of rats, as well as in the epiphyseal-metaphyseal parts of the tibial bones, whereas reduction of volume density of



of primary and secondary spongiosa was found only in hypokinetic rats. Both hypokinesia and suspension led to qualitative changes in the osteoblast population similar to those that occurred in the tibia. It should be noted that, unlike the latter, in hypokinetic rats there was also a decrease in number of osteoblasts. There was no change in number of osteoclasts in vertebral spongiosa of rats submitted to hypokinesia or suspension.

The findings warrant the belief that, in spite of the relatively short duration (7 days) of the experiment, it is possible to detect distinct signs of osteoporosis in rat spongiosa. Presence of signs of osteoporosis in the tibial bones was demonstrated in both experimental groups of rats. Thinning of the spongiosa of lumbar vertebrae was found only in hypokinetic rats. (In suspended rats, the decrease in volume density of primary and secondary spongiosa was statistically unreliable.) No signs of osteoporosis were demonstrable in compact bone of tibial diaphyses. Development of osteoporosis in the spongiosa of tibial bones and lumbar vertebrae is related primarily to inhibition of bone growth, as indicated by narrowing of the epiphyseal growth plate, zone of primary spongiosa and reduction in number of active osteoblasts. Still open is the question of whether development of osteoporosis during hypokinesia and suspension is attributable solely to inhibition of bone growth or whether there is concurrent intensification of its resorption, since increase in number of osteoclasts (one of the indirect signs of intensification of resorption) [9] was observed only in the tibial spongiosa but not in that of lumbar vertebrae. It should also be noted that, while the increase in number of osteoclasts is indicative of intensification of bone resorption, the obtained data are in contradiction to the view that intensification of bone resorption only occurs when a limb is deprived of its static function [1], since the number of osteoblasts in tibial bones increased, in this experiment, with both hypokinesia (static function retained) and suspension (no static function).

The changes in rat bones during hypokinesia and suspension were systemic, although their severity varied over a wide range in different bones and even in different parts of the same bone. Thus, distinct signs of osteoporosis were found in the spongiosa of tibial metaphyses, whereas no signs of osteoporosis were demonstrable in the compact substance of diaphyses of the same bones. The fact that the changes observed in rat bones with hypokinesia and suspension were in the same direction, as well as their relatively rapid onset, warrant the belief that an acute stress reaction in response to limiting the animals' movement [1, 3] or suspension in antiorthostatic position [10, 12] plays an appreciable role at the early stages of simulating weightlessness. It should be noted that the changes in vertebral spongiosa were less marked with suspension than hypokinesia, and this is consistent with the results of studies of adrenals and lymphoid organs of the same rats, which revealed that hypokinesia elicits more severe and longer lasting stress in rats than suspension.

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2]:616-008.939.15-39

LIPID PEROXIDATION IN RAT TISSUES WITH EXPOSURE TO ANTIORTHOSTATIC HYPOKINESIA,  
EXERCISE AND IMMOBILIZATION STRESS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21,  
No 6, Nov-Dec 87 (manuscript received 8 Dec 86) pp 39-43

[Article by A. Ye. Zezerov, S. M. Ivanova and A. S. Ushakov]

[English abstract from source] Lipid peroxidation in tissues of rats exposed to antiorthostatic hypokinesia ( $-15^{\circ}$ ) for 60 days, heavy-load exercise (swimming) and 2-hour immobilization was investigated polarographically. Antiorthostatic hypokinesia produced activation of free-radical lipid peroxidation in skeletal muscles, myocardium and plasma which reached a peak on hypokinesia day 3 and remained elevated by day 60. Exercise and immobilization applied during hypokinesia led to an accumulation of endogenous lipid peroxidation products in skeletal muscles and in the heart, although in a lesser degree. It is postulated that during hypokinesia lipid peroxidation is most probably activated due to the following factors: increased activity of the hormonal component of the sympathicoadrenal system, accumulation of excessive quantities of free fatty acids, and reduced activity of antioxidant enzymes.

[Text] The results of studies pursued in recent years revealed that processes of lipid peroxidation (LPO) play an important part in structural and functional modification of biological membranes, change in their physicochemical properties and permeability [1, 5, 6]. It was shown that activation of endogenous LPO, which is the key element in development of stress and ischemia [16], is a typical membrane mechanism of a number of pathological states [13].

Development of the hypokinetic syndrome is associated with change in physiological functions, appearance of systemic metabolic disorders, the nature and direction of which warrant the assumption that one of the mechanisms of their onset may be the appreciably increase in intensity of free-radical oxidation of membrane phospholipids, leading to accumulation of toxic products of oxidative destruction of lipids, functional impairment of membrane structures and corresponding change in cell metabolism [12, 21]. Results obtained from experiments with animals involving immobilization of various duration [3, 19, 20, 22-25] are indicative of the validity of this assumption.

It is known that long-term hypokinesia is associated with diminished adaptability, capacity for adequate tolerance to exercise and other extreme factors [12, 21] which, according to data in the literature [28-30], could in turn cause activation of lipoperoxidation processes.

Our objective here was to determine the effect of intensive exercise and an additional stress factor (immobilization stress) on LPO level in rats submitted to long-term antiorthostatic [head-down tilt] hypokinesia.

## Methods

Experiments were conducted with 80 male Wistar albino rats weighing 200-230 g. In accordance with the protocol, all animals were divided into the following groups: 1) control, 2) hypokinesia for 3 days, 3) hypokinesia for 60 days, 4) control + exercise, 5) hypokinesia for 60 days + exercise, 6) control + stress, 7) hypokinesia for 60 days + stress.

Antiorthostatic hypokinesia lasting a total of up to 60 days was produced by keeping the rats in plastic box-cages with negative angle of tilt of the head end in relation to the horizontal plane ( $-15^\circ$ ). Intact animals kept in the usual vivarium cages served as a control.

After 60-day hypokinesia, some of the control and experimental animals were submitted to maximum exercise load and 2-h immobilization stress. The physical load was produced by having the rats swim without weights in water at  $30^\circ\text{C}$  temperature until they were completely exhausted. Immobilization stress was produced by immobilizing the animals in prone position on a special platform [33]. The experiment was performed under conditions that precluded effects of extraneous stimuli.

Plasma isolated from the blood of decapitated rats and fixed in 0.02% alcohol solution of 2,6-ditertiary butyl-4 methylphenol (ionol), and frozen at  $-78^\circ\text{C}$  (dry ice); preparations of viscera (liver, heart, posterior group of crural muscles) were frozen at  $-196^\circ$  (liquid nitrogen). Lipids were isolated from blood plasma according to E. G. Bligh and W. J. Dyer [26] in the modification of M. Kates [11], and from organ tissues according to J. Folch et al. [31]. All procedures were performed at  $0\pm 4^\circ\text{C}$  temperature. The extractive mixture of solvents, methanol and chloroform, contained 0.001% ionol. Concentration of lipids was determined by gravimetry. Analysis of endogenous LPO products (lipid hydroperoxides and end products of dissociation) was performed on a P-8 Janagimoto (Japan) polarograph using a previously described method [8, 9]. The measurement results were expressed in relative units--nanoamperes/mg lipids.

## Results and Discussion

The data listed in the table show that restriction of mobility leads to activation of free-radical LPO processes in experimental animals. This is indicated by the accumulation of endogenous LPO products (lipid peroxides and end products of their dissociation) in the tested tissues throughout the hypokinetic period. Thus, on the 3d day of hypokinesia, levels of lipid hydroperoxides and end LPO products increased by 81.3 and 131.4%, respectively, in

skeletal muscles ( $p<0.050-0.01$ ), as compared to the control, by 49.3 and 87.2% ( $p<0.05$ ) in the myocardium, by 56.5 and 95.8% ( $p<0.05-0.01$ ) in plasma.

Levels of endogenous LPO products in rat tissues during antiorthostatic hypokinesia ( $M\pm m$ )

LPO parameters, nA/mg lipids	Tissue examined	Control	Hypokinesia, days	
			3	60
Hydroperoxide	Plasma	$8.36\pm 0.31$ ( $n=12$ )	$13.08\pm 2.21^*$ ( $n=9$ )	$7.12\pm 0.21$ ( $n=7$ )
	Myocardium	$6.06\pm 0.74$ ( $n=11$ )	$9.05\pm 0.89^*$ ( $n=8$ )	$7.01\pm 0.64$ ( $n=11$ )
	Skeletal muscles	$6.25\pm 0.66$ ( $n=6$ )	$11.33\pm 1.36^{**}$ ( $n=7$ )	$8.59\pm 0.23^{**}$ ( $n=7$ )
	Liver	$6.22\pm 0.87$ ( $n=6$ )	$6.10\pm 0.77$ ( $n=9$ )	$6.88\pm 1.35$ ( $n=8$ )
End products	Plasma	$39.52\pm 2.76$ ( $n=11$ )	$77.39\pm 8.83^{**}$ ( $n=9$ )	$60.54\pm 4.97^{**}$ ( $n=9$ )
	Myocardium	$26.90\pm 1.48$ ( $n=11$ )	$50.35\pm 8.41^*$ ( $n=7$ )	$36.14\pm 4.14$ ( $n=11$ )
	Skeletal muscles	$28.09\pm 5.28$ ( $n=6$ )	$64.99\pm 12.36^*$ ( $n=9$ )	$49.80\pm 3.84^{**}$ ( $n=7$ )
	Liver	$31.80\pm 5.39$ ( $n=7$ )	$31.49\pm 7.90$ ( $n=8$ )	$32.75\pm 7.56$ ( $n=8$ )

\*  $p<0.05$ .  
 \*\*  $p<0.01$ .

By the 60th day of hypokinesia, lipid peroxide content of the above-mentioned tissues decreased somewhat, but was still 37.4 and 77.3% (for hydroperoxides and end products, respectively;  $p<0.01$ ) above control values in skeletal muscles and by 53.2% higher (end products;  $p<0.01$ ) in plasma. The elevation of levels of LPO end products in the myocardium was statistically unreliable ( $p>0.05$ ) on the 60th day of hypokinesia.

According to the submitted material, the most marked change in intensity of free-radical LPO was observed in skeletal muscles, and this is consistent with the findings of other authors [25], stressing once more the leading role of the muscular system in development of the hypokinetic syndrome. The liver was the least affected: throughout the period of 60-day hypokinesia fluctuations of endogenous LPO product levels did not exceed 3-10% ( $p>0.05$ ). At the same time, the increase in lipid peroxide content of plasma showed a correlation to accumulation of LPO products in skeletal muscles and the myocardium at virtually all stages of hypokinesia. The latter circumstance enables us to view the intensity of LPO in plasma as an indirect indicator of activity of free-radical oxidative processes in body tissues.

Maximum exercise (swimming to exhaustion; Figure 1) had virtually no effect on LPO level in the myocardium. Conversely, there was significant increase in lipid hydroperoxides and LPO end products (by 92.7 and 97.6%, respectively;  $p<0.05$ ) in skeletal muscles, reaching values comparable to LPO level with antiorthostatic hypokinesia. Apparently, opposite functional states, with respect to motor activity (hypokinesia and intensive exercise) elicit changes in the same direction in free-radical LPO in muscles.



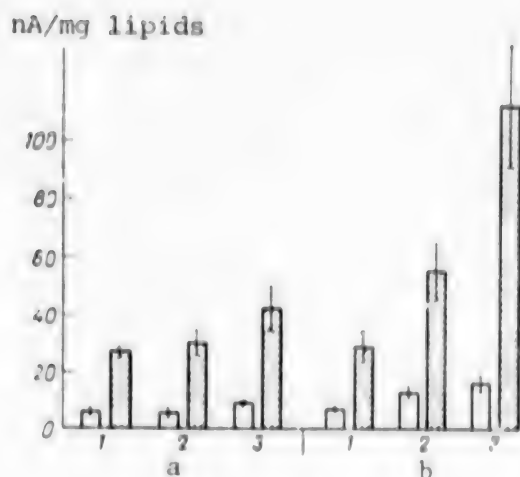


Figure 1.

Effect of intensive exercise on LPO level in animal tissues

- a) myocardium
- b) skeletal muscles
- 1) control
- 2) control + exercise
- 3) hypokinesia, 60 d + exercise

Here and in Figure 2:

White bars--lipid hydroperoxides,  
hatched--LPO end products

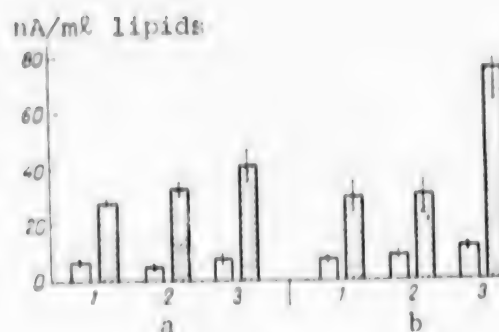


Figure 2.

Effect of immobilization stress on LPO in hypokinetic rats

- a) myocardium
- b) skeletal muscles
- 1) control
- 2) control + stress
- 3) hypokinesia, 60 d + stress

Other designations are the same as in Figure 1.

the myocardium and, particularly, skeletal muscles (see figure 1), exceeding the effect of hypokinesia alone with statistical reliability ( $p < 0.05$ ). Concentration of lipid peroxides (hydroperoxides and end products) increased by 41.9 and 56.9%, as compared to the control, in the myocardium and by more than 145 and 306%-- respectively ( $p < 0.01$ ) in skeletal muscles. The corresponding elevation of LPO level, as compared to the "control + exercise" group, constituted 27.3 and 105.5% ( $p < 0.05$ ).

The combination of prolonged hypokinesia and immobilization stress had an analogous but quantitatively less marked synergistic effect (Figure 2). In this case, maximum accumulation of lipid peroxides was recorded in skeletal muscles, where the levels of lipid hydroperoxides rose by 1.5-1.8 times, as compared to the control, and those of LPO products increased by 2.5-2.7 times ( $p < 0.05-0.01$ ).

LPO changes in the myocardium of rats in the "hypokinesia 60 days + stress" group were less marked. LPO end product content increased by 55.4% as compared to the control and by only 27.3%, as compared to the "control + stress" group ( $p < 0.05$ ). At the same time, one-time immobilization of intact rats led to elevation of LPO end product levels only in the myocardium (by 22%;  $p < 0.05$ ), without having an appreciable effect on concentration of lipid peroxides in skeletal muscles.

Analysis of the differentiated effect of antiorthostatic hypokinesia, intensive exercise, immobilization stress and combinations of these factors revealed that most of the above extreme factors unambiguously activate processes of

free-radical LPO in skeletal muscles. Lipid peroxide metabolism in the myocardium is more resistant to change in motor activity, and it is impaired chiefly by relatively brief exposure to stress factors (immobilization stress, 3-day hypokinesia). This is consistent with the findings of some authors [14] to the effect that the myocardium is more sensitive to acute stress than other tissues. A combination of several extreme factors (prolonged hypokinesia, intensive exercise, immobilization stress) leads to a marked synergistic effect and is associated with dramatic elevation of endogenous LPO levels. An analogous tendency was observed with a combination of hypokinesia and other aggravating factors [24].

Our findings are indicative of the fact that antiorthostatic hypokinesia, which acts as a prolonged neuroemotional stress factor, leads to increase in intensity of free-radical LPO processes in visceral tissues of laboratory animals, which is the most marked in the acute period of adaptation to unusual conditions. Changes in a similar direction were reported with clinostatic hypokinesia also [20, 25].

The stressor effect of hypokinesia is implemented with the participation of the system of neuroendocrine regulation. The high activity of the hormonal part of the adrenosympathetic system, which was demonstrated with hypokinesia [7], apparently leads to intensification of auto-oxidation of epinephrine to adrenochrome and, as a result, increased production of superoxide radicals ( $O_2^-$ )--initiators of free-radical oxidation of lipids in biological membranes [17, 27]. Mobilization of tissue lipases by catecholamines, which is observed under such conditions, leads to accumulation in plasma of free fatty acids, excessive concentrations of which have a significant deleterious effect on the cell membrane [32], impairing its structural and functional organization [17] and thereby increasing accessibility of phospholipid polyene acyls to oxidation by free radicals of oxygen. Activation of LPO under hypokinetic conditions is also instrumental in the possible decrease in activity of enzymes of antiradical protection of cells (superoxide dismutase, catalase), as well as shortage of the main antioxidant of membrane structures-- $\alpha$ -tocopherol [17, 18, 19]. Accumulation of phospholipid hydroperoxides and products of subsequent dissociation affects hydration of the cell membrane [2], viscosity and fluidity of the lipid bilayer [4]; it is associated with appearance of new permeability channels [10] and change in activity of enzymes involved in processes of energy-dependent ion transport [15]. The aggregate of these changes can cause appearance and development of systemic metabolic changes corresponding to the body's shift to a qualitatively new level of adaptation to altered environmental conditions.

Under such conditions, intense exercise and additional stress factors aggravate the functional and metabolic disorders elicited by hypokinesia, leading to dramatic activation of free-radical lipid oxidation in muscle tissue. The latter suggests that it is undesirable to use excessive physical and other stress-producing loads following long-term restriction of movement. Use of agents with antioxidant and selective metabolic action will reduce significantly the degree of hypokinesia-activated LPO [20] and improve several physiological functions.

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## ROLE OF CARBON DIOXIDE IN CORRECTION OF COAGULATION HEMOSTASIS UNDER HYPOXIC CONDITIONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (manuscript received 26 Aug 85) pp 43-47

[Article by G. D. Pak and V. S. Sverchkova]

[English abstract from source] In acute animal experiments coagulation hemostasis reactions and blood acid-base state were investigated when dogs were breathing hypoxic (10% O<sub>2</sub>) or hypoxic-hypercapnic (10% O<sub>2</sub>, 5% CO<sub>2</sub>) gas mixtures. When given the hypoxic mixture, activation of blood coagulation was accompanied by depression of anticoagulatory and fibrinolytic properties. These changes developed together with distinct hypoxemia, respiratory alkalosis and secondary metabolic acidosis. When given the hypoxic-hypercapnic mixture, no hypercoagulation occurred which can be explained by higher (than on the hypoxic mixture) p<sub>a</sub>O<sub>2</sub>, lack of disorders in acid-base equilibrium and in oxygen supply. It is believed that the ability of carbon dioxide to maintain relative normocoagulation when added to the hypoxic mixture is one of the factors that increase tolerance to hypoxia.

[Text] Previous investigations demonstrated that hypoxia, which enhances general coagulability of blood and diminishes its anticoagulation and fibrinolytic activity [6, 9, 13], creates conditions that facilitate formation of a pretbrombotic state. For this reason, use of antihypoxic agents is of therapeutic and preventive importance. Carbon dioxide is an effective means of enhancing tolerance to hypoxia. Its properties of activating the system of delivery of oxygen to tissues, affecting metabolic processes and function of regulatory systems [1, 2, 4, 7] enhance the reserve capabilities of the body and attenuate the effect of hypoxia. At the same time, the question of involvement of the hemostasis system in adaptive reactions to the combination of hypoxia and hypercapnia has not been sufficiently explored.

Our objective here was to examine the clotting and fibrinolytic properties of blood under hypoxic and hypoxic-hypercapnic conditions, analyze blood-clotting reactions as a function of level of hypoxemia and acid-base equilibrium (ABE) of blood.



## Methods

Acute experiments were performed on 23 mongrel dogs weighing 5-12 kg under hexenal anesthesia, breathing a hypoxic (10% O<sub>2</sub> in nitrogen) and hypoxic-hypercapnic (10% O<sub>2</sub>, 5% CO<sub>2</sub> in nitrogen) mixture. Blood samples were drawn from the lateral branches of the femoral arteries before using the breathing mixtures (while breathing atmospheric air) and after 25-min use of one of the mixtures, and we used silicone-coated dishes.

To examine coagulation hemostasis we used the following tested methods [5, 8]: silicone time for plasma after Beller and Graeff; kaolin time for plasma rich and poor in thrombocytes after Hattersley in the modification of Z. S. Barkagan; test for demonstration of cold activation of kallikrein "bridge" between factors XII and VII after Stormorken et al.; thrombin time and free heparin after E. Sirmai; antithrombin III activity after Abildgaard et al.; ethanol test after Godal et al.; fibrin-stabilizing factor activity after Baluda in the modification of G. V. Andreyenko; fibrinogen concentration and fibrinolytic activity after Bidwell in the modification of G. V. Andreyenko; nonenzymatic fibrinolysis after Bidwell (with addition of  $\epsilon$ -aminocaproic acid); calculation of index of contact activation range (ICAR) and index of thrombocyte activation release (ITAR) after G. F. Yeregin et al.; calculation of indexes of nonenzymatic and enzymatic fibrinolysis after N. A. Avdeyenko.

Oxygen (p<sub>a</sub>O<sub>2</sub>) and carbon dioxide (p<sub>a</sub>CO<sub>2</sub>) tension and pH of arterial blood were determined using a microgas analyzer. ABE parameters were calculated using Sigaard-Andersen nomograms. The material was processed by the method of variation statistics after Student.

## Results and Discussion

Breathing the hypoxic mixture elicited significant increase in clotting potential (Table 1). Silicone time with minimal contact activation increased by 30.5%, kaolin time with maximum contact activation of thrombocyte-rich and thrombocyte poor plasma decreased by 27.2 (17.2 s) and 27.9% (33.3 s), respectively. The more marked reduction in clotting time for thrombocyte-poor plasma, as well as ITAR (by 6.1%), which reflects thrombocytic activation of blood clotting, leads us to assume that a phospholipid component of platelets appears in plasma. The 6.7% decline of ICAR confirms the presence of a hypercoagulation change under hypoxic conditions due to contact and phospholipid activation of blood-coagulation triggering mechanisms.

We demonstrated a reliable decrease (by 25.2%) in heparin activity and a tendency toward decline in activity of the strongest anticoagulant, antithrombin III.

The test for demonstration of the kallikrein "bridge" between factors XII and VII showed stable reduction of plasma clotting time after cold activation, by a mean of 5 s without, however, sufficient reliability. The ethanol test remained negative.

There was significant increase, by 32.4%, in fibrinogen concentration. Indexes of nonenzymatic and enzymatic fibrinolysis decreased by 46 and 40.9%, respectively.

Table 1. Blood clotting activity when breathing hypoxic and hypoxic-hypercapnic mixtures ( $M \pm m$ )

Parameter	Baseline data	Hypoxic mixture	Hypoxic-hypercapnic mixture
Silicone time, s	200,1 $\pm$ 4,6	139,2 $\pm$ 5,9*	206,2 $\pm$ 7,2
Kaolin time for thrombocyte-rich plasma, s	63,1 $\pm$ 2,6	45,9 $\pm$ 3,0*	62,5 $\pm$ 3,5
Same for thrombocyte-poor plasma, s	119,4 $\pm$ 7,8	86,1 $\pm$ 5,0*	120,2 $\pm$ 10,3
ICAR, %	69,8 $\pm$ 1,0	65,1 $\pm$ 1,7*	66,8 $\pm$ 2,7
ITAR, %	48,6 $\pm$ 2,3	45,6 $\pm$ 3,2	50,7 $\pm$ 3,5
Thrombin time, s	18,4 $\pm$ 0,4	16,2 $\pm$ 0,5**	17,1 $\pm$ 0,8
Free heparin, s	8,6 $\pm$ 0,4	6,4 $\pm$ 0,3*	6,3 $\pm$ 0,4*
Antithrombin III activity, %	103,2 $\pm$ 7,9	92,6 $\pm$ 10,3	119,4 $\pm$ 7,8
Fibrinogen, mg%	319,9 $\pm$ 17,4	423,5 $\pm$ 37,5***	401,0 $\pm$ 32,7**
Factor XIII activity, s	62,2 $\pm$ 5,3	56,6 $\pm$ 10,5	44,4 $\pm$ 6,4**
Ethanol test	—	—	—
Nonenzymatic fibrinolysis index	1,04 $\pm$ 0,1	0,53 $\pm$ 0,1***	1,14 $\pm$ 0,3
Fibrinolysis index	0,88 $\pm$ 0,1	0,52 $\pm$ 0,1*	1,24 $\pm$ 0,1

Here and in Table 2: \* $p < 0.001$  \*\* $p < 0.01$  \*\*\* $p < 0.02$  \*\*\*\* $p < 0.05$

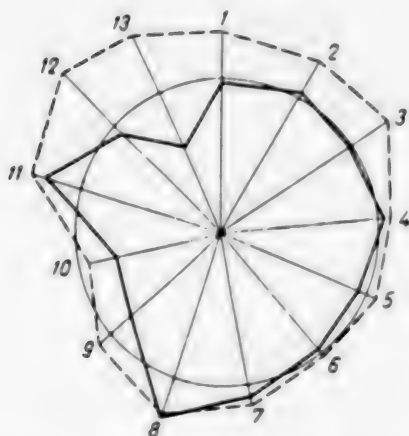
Table 2. Gas composition and ABE of blood when breathing hypoxic and hypoxic-hypercapnic mixtures ( $M \pm m$ )

Parameter	Baseline data	Hypoxic mixture	Hypoxic-hypercapnic mixture
$P_{aO_2}$ , mm Hg	79,2 $\pm$ 2,1	27,6 $\pm$ 1,8*	45,3 $\pm$ 1,9*
$P_{aCO_2}$ , mm Hg	38,4 $\pm$ 0,8	24,4 $\pm$ 0,7*	39,7 $\pm$ 0,8
pH	7,399 $\pm$ 0,007	7,436 $\pm$ 0,014	7,368 $\pm$ 0,008**
BD, mmol/l	0,32 $\pm$ 0,50	7,26 $\pm$ 0,90*	2,13 $\pm$ 0,42***

The observed changes in blood clotting developed in the presence of intensified removal of carbon dioxide from blood ( $P_{aCO_2}$  dropped by 36.5%) and rise of pH. The dramatic drop of blood oxygen tension (by 75.2%) caused inconsistency between levels of delivery and utilization of oxygen by tissues, while the increase in buffer base deficit (BD) was indicative of appearance of incompletely oxidized metabolic products in blood (Table 2).

It is known that rise of pH in excess of 7.4 [11], as well as hypoxemia and alkalosis [12], elicit an increase in thrombocyte reactivity. The opinion is held [3] that gas alkalosis, even under normoxic conditions, causes elevation of prothrombin index and acceleration of blood clotting time.

Perhaps, the increase in pH and change in ABE in our experiments are the causes of formation of an adverse reaction of the hemostasis system under hypoxic conditions, when activation of blood clotting is associated with depression of its anticoagulant and fibrinolytic elements.



Comparative evaluation of blood-clotting system when inhaling hypoxic and hypoxic-hypercapnic mixtures

Boldface and dash lines--hypoxic-hypercapnic and hypoxic conditions, respectively. Circular line--baseline values (breathing atmospheric air), 100%

- 1) silicone time
- 2) kaolin time for thrombocyte-rich plasma
- 3) kaolin time for thrombocyte-poor plasma
- 4) ICAR
- 5) ITAR
- 6) test for demonstration of cold activation of kallikrein bridge between factors XII and VII
- 7) thrombin time
- 8) free heparin
- 9) antithrombin III activity
- 10) factor XIII activity
- 11) fibrinogen concentration
- 12) nonenzymatic fibrinolysis
- 13) fibrinolytic activity

Outside of circle--hypercoagulation parameters; inside circle--hypocoagulation parameters

time ( $p < 0.001$ ), kaolin time for thrombocyte-rich ( $p < 0.001$ ) and poor ( $p < 0.05$ ) plasma, antithrombin III activity ( $p < 0.05$ ), as well as fibrinolytic activity ( $p < 0.001$ ), and progressive decline in activity of fibrin-stabilizing factor (see Figure).

In the next series of experiments, addition of 5%  $\text{CO}_2$  to the hypoxic mixture prevented removal of carbon dioxide from blood and stabilized  $\text{p}_a\text{CO}_2$  and pH at close to baseline levels (see Table 2). Blood oxygen tension dropped 32.6% less than when breathing the purely hypoxic mixture. Judging from the insignificant change in BD, this provided for better delivery of oxygen to tissues. Against this background, we failed to demonstrate reliable differences in the blood-clotting system according to several basic parameters, as compared to baseline data (see Table 1). Only the increase in fibrinogen concentration (by 25.3%), decrease in activity of fibrinase (by 28.4%) and heparin (by 26.6%) were reliable and retained the same direction as with use of the hypoxic mixture. Consequently, the changes in these parameters are the most sensitive and stable response to drop of blood oxygen tension within the range we studied ( $\text{p}_a\text{O}_2$  47-25 mm Hg), and they depended little on pH,  $\text{pCO}_2$  or intensity of oxidative processes in tissues.

It should be noted that depression of non-enzymatic fibrinolysis is not demonstrable with addition of carbon dioxide to the hypoxic mixture. This is particularly important under hypoxic conditions, since heparin, which forms a complex with epinephrine, neutralizes the transmitter of the sympathetic nervous system and thus prevents activation of blood-clotting triggering mechanisms. In addition, there is a tendency toward increase in fibrinolytic activity of blood, which is viewed by some authors as a protective mechanism against pulmonary edema in the presence of hypoxia [10].

A comparison of blood coagulation system with inhalation of hypoxic and hypoxic-hypercapnic mixtures revealed reliable differences in parameters of silicone

The capacity, which we demonstrated, of carbon dioxide to maintain a state of relatively normal coagulation when added to a hypoxic mixture is apparently one of the factors that enhances resistance to hypoxia.

The results of our experiments enable us to assume that regulation of blood clotting under hypoxic conditions is consistent with tissular metabolism. Thus, tissue hypoxia and respiratory alkalosis (when breathing a mixture containing 10% O<sub>2</sub> in nitrogen) is correlated to activation of coagulant properties and depression of anticoagulant properties of blood. Addition of carbon dioxide (5% CO<sub>2</sub>) to the hypoxic mixture is instrumental not only in maintaining normocapnia in blood, but improving oxygenation of tissues [1, 4, 7], and it prevents development of metabolic acidosis. In this case, there are insignificant changes in the system of coagulation hemostasis, and there are no signs of hypercoagulation. Consequently, the hypoxic stimulus acquires its relevance as a signal (activating) for the blood-clotting system only at levels that lead to overt impairment of delivery of oxygen to tissues and changes in ABE.

Thus, it was established that addition of carbon dioxide to a hypoxic mixture creates conditions that preclude activation of blood clotting under hypoxic conditions. These data are rather promising, with regard to development of nonspecific antihypoxia agents. It is imperative to give the closest scrutiny to analysis of ABE and correction of its disturbances in the detection and treatment of hypercoagulation states.

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EFFECT OF CARDIOACTIVE COMPOUNDS ON RAT MYOCARDIUM ACTOMYOSIN FOLLOWING EXPOSURE TO ACCELERATIONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (manuscript received 15 Jul 86) pp 47-49

[Article by M. A. Kayfadzhyan and B. A. Tikunov]

[English abstract from source] It has been shown that during centrifugation the modulating effect of cardioactive compounds, particularly adrenalin and obsidan, varies in similarity to that of Ca ions. The reactivity of the native actomyosin complex of the heart of white rats to such agents declines during centrifugation. This may be associated with changes in regulatory protein components through which the modulating effect of the above compounds is mediated. Differences in the reactivity to adrenalin and obsidan that persist after 2-month readaptation can be attributed to the heterogeneous recovery of properties of individual subunits of regulatory proteins.

[Text] It is known that ruptures and separations of insertion disks and damage to cell membranes are observed in the presence of myocardial pathology [3, 6]. This is the cause of their increased permeability to ions and diverse compounds in the intercellular space. For this reason, we cannot rule out the possibility that, with forced exercise, some cardioactive compounds can penetrate into the saroplasm and have a direct effect on the myofibrillar system of cardiomyocytes.

It was previously established that  $Mg^{2+}$ -ATPase of native actomyosin (NAM) of the myocardium can be activated by certain cardioactive compounds [9, 10]. The agent of these agents, which is comparable to the modulating effect of Ca ions, and their capacity for binding with regulatory proteins of the actomyosin complex [4] led to the assumption that  $Ca^{2+}$ -binding proteins can serve as adrenoreceptors, in addition to the regulatory subunit of adenylate cyclase [15].

However, since there can be an appreciable change in  $Ca^{2+}$  regulation of complex-forming and enzymatic activity of actomyosin when the mode of myocardial function is changed [7], it was of particular interest to investigate reactivity of actomyosin to cardioactive agents when there is gradual increase in exercise load ("adaptation period") as well as during recovery.

## Methods

Experiments were performed on male white rats weighing 180-200 g (total of 90 animals). The animals were divided into three groups: the first consisted of control animals that were not submitted to any stimuli and maintained in the vivarium under the same conditions as experimental groups. The second and third groups consisted of rats submitted to periodical gravity accelerations on a centrifuge with 160 cm radius of rotation. During rotation, the animals were immobilized in special chambers which permitted exposure to centrifugal force in the head-tail direction. The gradient of build-up of accelerations constituted 0.08 G/s. The animals were submitted to accelerations of +5 Gx for 15 days. On the 1st day, they were on the centrifuge for 5 min, and the time was increased daily by 5 min each day thereafter. After 5 days, the animals were rotated for 30 min daily at the same time of day. The 2d group of rats ("adaptation") was decapitated under ether anesthesia on the 2d day after termination of exposure. The 3d group--"recovery"--was examined 2 months after termination of exposure to accelerations.

NAM was recovered from the left ventricle by the method described in [13], and desensitized actomyosin (DAM) was recovered from the NAM suspension [14]. Purity of protein preparations was checked by electrophoresis [16] and spectrophotometry according to  $D_{280}/D_{260}$  ratio; protein concentration was measured by the Lowry method.

The superprecipitation (SPP) reaction and actomyosin ATPase were studied using a previously described method [8] in a medium containing 20 mM tris-HCl, 0.15 M KCl, 0.025 mM  $MgCl_2$ , 0.25 mM ATP, 0.25 mg/ml protein and 4 mM DTT [dithiothreitol?], pH 7.35, at a temperature of 25°C.

The effect of  $Ca^{2+}$  admixture was ruled out by addition of 1 mM EGTA [typo for EDTA?] to the incubation medium.

Extent of cooperation of ATPase reaction with SPP reaction ( $h$ ) was calculated graphically using Hill's equation.

## Results and Discussion

In concentrations of  $10^{-9}$ - $10^{-6}$  M, epinephrine activates the rate of SPP and ATPase reaction of NAM in control rats, the maximum activating effect on both rate of SPP and level of  $Mg^{2+}$ -ATPase being reached with  $10^{-8}$  M epinephrine (Figure 1a, b). The change in SPP rate is characterized by an overt sigmoid pattern and positive cooperation ( $h = 1.9$ ), which coincides with previously obtained results of studies of  $Ca^{2+}$  reactivity of NAM, where  $h = 1.5$  [2].

A decline in rate of  $Mg^{2+}$ -ATPase and SPP reaction, by 56 and 50%, respectively, is observed under the effect of accelerations ("adaptation group") with  $10^{-8}$  M epinephrine. Under these conditions, there was also decrease in  $Ca^{2+}$  reactivity of NAM preparations [7].

It is not difficult to notice that, 2 months after exposure to accelerations ("recovery group") there was a tendency toward restoration of baseline values

for the parameters studied. There was restoration of sigmoid pattern of kinetic curves, level of half-maximum activation of ATPase and rate of NAM SPP.

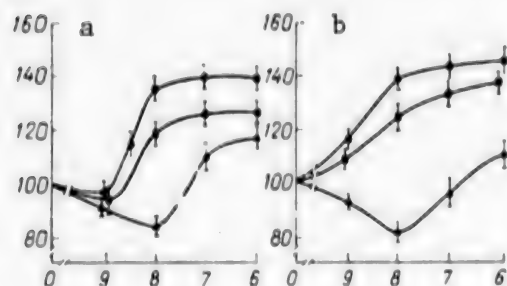


Figure 1.

Change in  $V_{SPP}$  (a) and  $Mg^{2+}$ -ATPase (b) of myocardial NAM in control (●), adaptation (▼) and recovery groups (■) of animals in presence of different concentrations of epinephrine in medium

X-axis,  $\log M^{-1}$  epinephrine; y-axis, here and in Figure 2, relative changes in  $V_{SPP}$  and  $Mg^{2+}$ -ATPase (%)

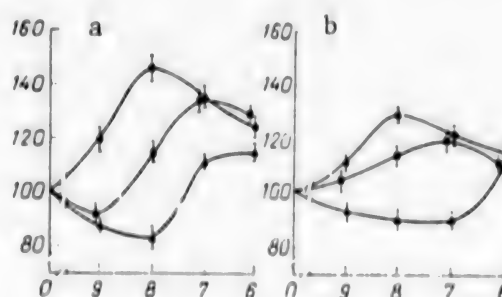


Figure 2.

Changes in  $V_{SPP}$  (a) and  $Mg^{2+}$ -ATPase (b) of myocardial NAM in control (●), adaptation (■) and recovery (▼) groups of animals in the presence of different concentrations of obsidan in medium

X-axis,  $\log M^{-1}$  obsidan

The findings were somewhat different with use of obsidan [propranolol hydrochloride], which is a  $\beta$ -blocker

(Figure 2a, b). The kinetic curves of concentration as a function of rate of SPP and NAM ATPase reaction are bell-shaped. As was the case of interaction with epinephrine, the strongest activating effect of obsidan on control NAM preparations was observed with a concentration of  $10^{-8}$  M. However, the effect is reversed with accelerations, and shifts the maximum saturating concentration in the direction of lower values,  $10^{-7}$  in the case of SPP and  $10^{-6}$  M for the ATPase reaction.

The curve of  $Mg^{2+}$ -ATPase function is characterized by inhibition over the entire range of tested concentrations of the  $\beta$ -blocker, with the exception of  $10^{-6}$  M, whereas SPP rate already increased with  $10^{-7}$  M. Otherwise, these two parameters changed in a similar manner. Interestingly enough, during recovery the shift in maximum ATPase response activity and rate of SPP observed with  $10^{-7}$  M obsidan did not revert to the baseline. This is consistent with data to the effect that there is nonuniform restoration of different components of modulator proteins of the troponin-tropomyosin complex in the recovery period [1, 5]. Evidently, the dissimilar nature of restoration of concentration dependence of rate of SPP and ATPase reaction of NAM in the presence of epinephrine and obsidan is related to the modulating effect of different troponin subunits.

According to Figure 3, epinephrine and obsidan do not have an activating effect on DAM  $Mg^{2+}$ -ATPase due to absence in it of  $Ca^{2+}$ -binding component of the troponin-tropomyosin complex.  $Ca$  ions also have no effect on DAM  $Mg^{2+}$ -ATPase [11].

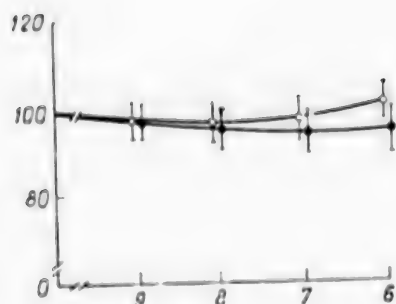


Figure 3.  
Changes in myocardial DAM  $Mg^{2+}$ -ATPase in control group of animals in the presence of different concentrations of epinephrine (●) and obsidan (○)  
X-axis,  $\log M^{-1}$  cardioactive agent; y-axis, relative changes in  $Mg^{2+}$ -ATPase (%)

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Thus, our findings warrant the belief that the modulating effect of cardioactive compounds on the actomyosin complex, in the case of an increased physical load on the heart, changes similarly to the effect of Ca ions.

The decrease in reactivity to cardioactive agents in the adaptation period, just like that of Ca ions, is related to changes in regulatory protein components of the native actomyosin complex, through which the modulatory effect of these compounds occurs. Residual differences in reactivity of actomyosin to cardioactive compounds, which persist after a 2-month recovery period, are apparently attributable to incomplete restoration of properties of regulatory proteins.

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MORPHOLOGICAL MANIFESTATIONS OF PRIMATE ADAPTATION TO ORTHOGRADE STATICS  
AND WALKING ERECT

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21,  
No 6, Nov-Dec 87 (manuscript received 29 Aug 86) pp 51-54

[Article by G. S. Belkaniya, Yu. N. Kurochkin, A. S. Rakhmanov, K. V.  
Simavonyan, V. A. Dartsmeliya, A. N. Demin and V. Ye. Filenko]

[English abstract from source] Using lower primates (rhesus monkeys) who were experimentally transformed to bipeds, we examined morphological signs of their musculoskeletal adaptation to the upright walking pattern. It was found that the bipeds developed typical characteristics of the upright posture: complete erectness of the torso and legs and noticeable enhancement of lumbar lordosis. The maximum deviation of the lumbar arch in the bipeds ( $10.0 \pm 0.8$  mm) was significantly greater than in the controls ( $1.5 \pm 0.5$  mm). This was accompanied by distinct hypertrophy of leg muscles, primarily extensors, and increased mineral density of the tibia (by 38%), fibula (by 14%) and metatarsus (by 23%). This was also followed by slight hypotrophy of biceps and triceps muscles and the large muscle of the thorax. Forearm muscles and bone mineral content were unchanged. The data obtained give evidence that the genetic program of orthograde statics and erect posture which is typical of the entire primate order can find phenotypical realization in lower primates.

[Text] Comparative anatomical studies yielded data concerning the basic phylogenetic direction of morphological transformations in the course of evolutionary and ontogenetic formation of orthograde walking in hominids [4, 5]. However, these sparse paleontological data enable us to reconstruct only in the first approximation the transitional forms in the process of formation of upright walking [1, 3].

As shown by previous studies [2], the model of experimental bipedia in primates is one of the promising approaches to answering these questions. Our objective here was to demonstrate the morphological manifestations of postural adaptation of the skeletomuscular system to the gravity factor in orthograde statics and upright walking in primates.

## Methods

Studies using the developed experimental biped model [2] were pursued on 6 experimental and 10 control *Macaca rhesus* males 3.5-4 years of age. Somatometry was performed on ketamine-anesthetized animals by standard anthropometric methods adjust for measurements on primates, using standard anthropometric instruments. X-rays of the skeletal bones were taken on nembutal-anesthetized monkeys, under standard immobilization conditions, as well as on nonanesthetized animals standing freely on a primatological stand, using a TUR-800 x-ray unit.

Bone minerals in the distal elements of the skeleton were measured by the method of gamma-photon absorptiometry. The experimental protocol involved successive scanning of selected parts of the limbs with a thin beam of  $\gamma$ -quanta (3 mm in diameter), followed by calculation of absorption of  $\gamma$ -quanta by bone. First a rubber cuff with distilled water was placed on the animal's limb, which enabled us to avoid measurement errors related to soft tissue scanning.

We determined the mineral content in milligrams per square centimeter of scanned surface for each area scanned. In each monkey, we determined mineral density of the tibia and fibula at 50 and 75% of its length (from the calcaneus), proximal segment of the metatarsus, as well as radius and ulna at 15 and 50% of their length (from the styloid process of the ulna). Measurements were taken for limbs on both sides. According to the literature, reproducibility of results with this method is at least 2% for man [7], and it is 3-5% in studies of primates [6].

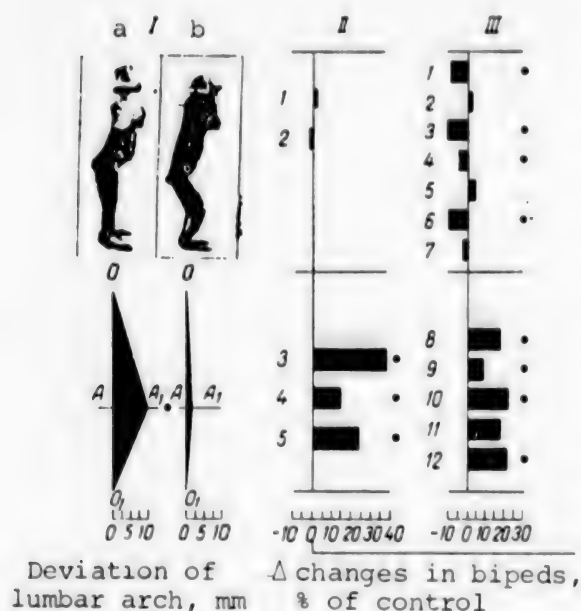
## Results and Discussion

For the first months after switching the monkeys to constant orthograde conditions, typical signs of erect position appeared in their posture. There was distinct evidence of complete straightening of the torso and lower limbs in biped animals. The posterior outline of the body clearly showed typical flexures of the spine--thoracic kyphosis and lumbar lordosis. In the biped monkeys, the typical features of orthograde position were retained and became even more prominent when walking and running.

General analysis of x-rays of the skeleton of bipeds and control animals revealed the most marked anatomical differences in geometry of vertebrae. The bipeds showed a marked tendency toward greater lordosis, which is a typical distinction of upright-walking man. For objective evaluation of severity of lumbar lordosis, we measured making deviation of the lumbar arch (MDLA). Measurements were taken in the following manner: we connected with a straight line ( $OO_1$ ) between the posterosuperior margin of the first lumbar and lateroinferior margin of the 7th lumbar vertebra on a lateral x-ray of the lumbar spine and restored a perpendicular line ( $AA_1$ ) in relation to the farthest point of the spinal arch (at the level of the 4th-5th vertebrae). The height of the perpendicular line (in mm) is an indicator of MDLA, reflecting extent of lordosis in the lumbar spine.

Mean MDLA in control monkeys kept under ordinary conditions of motor activity constituted 1.5 0.5 mm. After 8 months under orthograde conditions, it

increased in biped monkeys to  $4.5 \pm 0.8$  mm ( $p < 0.02$ ), and after 11-12 months, to  $10.0 \pm 0.9$  mm ( $p < 0.001$ ). In the same observation time, MDLA underwent virtually no change in control animals.



Main manifestations of postural adaptation of the musculoskeletal system of experimental biped monkeys

Formation of typical signs of body stance and deflections of the spine; change in mineral density of bones and muscles of upper and lower extremities (characteristics of control animals taken as 100%). See text for the rest of the designations.

immobilizing placement of the torso in the lumbar region, in the plane that is closer to the horizontal plane, the angle between long axes of the spine and femur virtually failed to increase. Such a contrived position of the spine in rats was associated with a specific load on the ligament and articulation system, which led to development of degenerative changes, which were particularly marked in the intervertebral disks. It is not by chance that biped rats were used as a model of spondyloarthrosis deformans [8]. The absence of such changes in monkeys serves as additional evidence of the evolutionary readiness of this animal species to change to semi-erect and orthograde statics, which determined several appropriate morphological and functional transformations in the skeletomuscular system in the course of physical development of primates under experimental biped conditions for a period of several years.

It should be borne in mind that positioning for the x-rays could have affected degree of lordosis in anesthetized animals. There could be contrived decrease or increase in lordosis. For this reason, skeletal x-rays were taken with the monkeys standing freely in the primatological device (see Figure). Differences in vertebral geometry between control animals and bipeds were demonstrable just as distinctly, and the dependence of extent of lumbar lordosis on contrived positioning of the monkeys was eliminated. In bipeds, deviation of the arch constituted  $10.0 \pm 0.8$  mm, whereas in control animals this deviation did not exceed 1.5 mm. Aside from differences in degree of lumbar lordosis, x-rays of the monkeys standing freely revealed distinct differences between bipeds and control animals also in correlation between the main elements of the static skeleton and limbs. While control animals stood on half-flexed legs with the torso tilted forward, the bipeds showed distinct verticalness of the trunk, with vertical alignment of the long axis of the femur and spine, which is inherent in human orthograde statics.

It should be noted that we failed to observe degenerative changes in biped monkeys 8, 11-12 months and 2 years after the experiment, in contrast to biped rats [8]. In rats, due to the

The specific load on the pelvis and legs in upright walking was associated with marked hypertrophy of pelvic and lower-limb muscles. There was distinct evidence of dissociation of dynamic changes in volumes of the upper and lower limbs. It should be noted that, while forced limitation of motion of the upper limbs for the first month of biped conditions was associated with relative muscular hypotrophy, thereafter there was manifestation of positive dynamics of changes in arm and forearm volume. In addition, we observed significant increase in muscle mass of the thigh and lower leg, by 22 and 12%, respectively [2]. Further exposure to experimental orthograde conditions of the monkeys led to intensification of dissociation between development of muscles of the upper and lower extremities due to predominant hypertrophy of extensors (increase in thickness of the femoral quadriceps and gastrocnemius, in addition to increase in circumference of the thigh and leg) and moderate hypotrophy of shoulder girdle muscles, more marked in flexors (biceps, large thoracic muscle). At the same time, there was no appreciable difference in development of forearm muscles, as compared to control animals.

Mineral density of bone (mg/cm<sup>2</sup>) in control animals (C) and biped monkeys (B) according to results of  $\gamma$ -photon absorptiometry

Measurements	Lower extremities						Upper extremities			
	tibia		fibula		metatarsus		ulna		radius	
	C	B	C	B	C	B	C	B	C	B
Total density	751 ±36	1039 ±28*	603 ±28	686 ±22**	504 ±20	620 ±37**	615 ±21	622 ±25	600 ±11	585 ±30
Right extremity	652 ±55	1036 ±42*	625 ±42	708 ±37	519 ±33	629 ±43**	632 ±25	606 ±34	595 ±28	586 ±49
Left extremity	793 ±39	1039 ±40*	584 ±38	663 ±22	486 ±17	571 ±32***	593 ±37	638 ±44	598 ±8	584 ±46
M/3	809 ±63	1132 ±27*	655 ±26	717 ±20	—	—	672 ±19	710 ±15	650 ±20	666 ±21
P/3	708 ±36	946 ±28*	588 ±39	654 ±36	—	—	588 ±27	534 ±36	542 ±14	504 ±13

M/3--middle 3d of bone, P/3--proximal 3d. \* $p < 0.001$ , \*\* $p < 0.01$ , \*\*\* $p < 0.05$

These data are indicative of a specific direction of plastic processes in skeletal muscles of biped monkeys. The more marked functional load on the lower limbs with upright walking stimulates increase in muscle mass of the pelvic girdle of the axial skeleton and lower extremities, which is more marked in antigravity muscles (extensors of the thigh and lower leg). There is also more marked increase in volume of gluteus muscles. In addition, limitation of crawling by biped monkeys with use of the arms had a more marked effect on flexor muscles of the shoulder girdle, without affecting muscles of the forearm.

Definite orientation was also noted in biped monkeys with respect to changes in mineral density of bone in the distal parts of the skeleton (see Table). First of all, we must mention the marked increase in mineral density of the tibia ( $p < 0.001$ ) and fibula ( $p < 0.01$ ) in bipeds after walking erect for 1 year. Mineral density of the tibia increased relatively more (by 59% for the right, 31% for the left and by a mean of 38% for both bones), as compared to the fibula (by 13, 14 and 14%, respectively). There was also significant increase in mineralization of the metatarsal bones (by 23%). At the same time, mineral density of the ulna and radius was the same throughout the same observation period in control and biped animals.



Dissociation of changes in mineralization of crural and forearm bones under control conditions and with experimental bipeds reflects alteration of bone in the lower extremities due to increase in axial load when walking upright. Significant difference in mineral content of the tibia and fibula is more evidence of this, whereas in control animals these differences are much less marked. Evidently, this is related to the fact that with upright walking the main weight load is distributed over the tibia which also becomes more consolidated under these conditions. Greater mineralization ( $p < 0.01$ ) was observed in the middle third of the tibia ( $1132 \pm 27 \text{ mg/cm}^2$ ) than the proximal segment ( $964 \pm 28 \text{ mg/cm}^2$ ) in accordance with the difference in distribution of mechanical load on this bone in bipeds. At the same time, these parts of the tibia do not differ in mineral content in control animals. Equalization of the mechanical load on both lower extremities was manifested by disappearance, in the bipeds, of lateral asymmetry in density of the left and right crural bones, whereas in control animals mineral content was somewhat higher in the left tibia than the right. It is important to note that mineralization of the tibia became virtually the same in biped monkeys as in man [7].

Thus, a relative increase in gravity load on the axial skeleton under experimental biped conditions leads, in monkeys, to interrelated changes in the muscular and bone systems of the skeleton, which are particularly marked in the pelvis and lower limbs. No appreciable differences in muscle mass and density of bones of the upper extremities between experimental and control animals were demonstrable, which is indicative of retention of the necessary conditions for normal development of upper extremities in biped monkeys. On the whole, it should be noted that orthograde conditions in monkeys stress the typical features of orthograde statics in the axial skeleton and enhance manifestations of transverse asymmetry of the skeletal and muscular systems. The main direction of this asymmetry is predominant development of the skeleto-muscular system of the pelvis and lower limbs, which is inherent in orthograde man and is one of the basic elements of his constitutional species-specific distinctions.

The above data are indicative of rather marked phenotypic realization in monkeys of the genetic program common to the order of primates for implementation of orthograde statics and upright walking, with regard to the main morphological and functional components: virtually totally vertical axial skeleton, formation of typical vertebral geometry (lumbar lordosis, thoracic kyphosis) without degenerative manifestations referable to bone and connective-tissue elements of the spine; the directional dissociation of changes in mineral content of bones and muscle mass of the upper and lower extremities with increase in transverse asymmetry of their development; with reference to the skeleto-muscular system of the pelvic girdle and lower extremities, predominant changes in bones carrying the greater weight load and antigravity muscles, and, finally, the very possibility of complete assimilation of all the complicated forms of locomotion (walking, running, jumping and crawling) under orthograde conditions.

The demonstrated morphological manifestations of adaptation of monkeys to upright walking constitute additional information concerning the form-producing influence of earth's gravity on animals, and they delineate future opportunities of experimental investigations on the model of biped monkeys, not only in the area of gravity biology, physiology of stance and motion, but morphology and physiology of anthropogenesis.



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EFFECT OF EXOGENOUS CARDIOSYNCHRONIZED COUNTERPULSATION ON HUMAN REGIONAL AND CENTRAL HEMODYNAMICS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (manuscript received 20 Nov 86) pp 54-58

[Article by Kh. Kh. Yarullin, T. D. Vasilyeva and I. P. Neumyvakin]

[English abstract from source] Variations in regional and central hemodynamics of man under the influence of exogenous contrapulsation (ECP) were examined rheographically. In recumbent subjects, ECP increased cerebral pulse blood filling, particularly of the vertebrobasilar system. It appears that the significant increase of the tone of arterioles and veins may be important when it becomes necessary to correct cerebral circulation in the situations that may lead to brain ischemia. It has been found that in the orthostatic tilt test ECP can be an effective tool for enhancing brain and lung blood supply and increasing stroke volume, cardiac output and blood pressure. This may help maintain homeostasis and improve orthostatic tolerance. The observation that ECP may facilitate blood supply of the vitally important organs, specifically brain, enables us to recommend this procedure to be used at an early stage of readaptation after simulation or real space flights.

[Text] At the present time, several methods have been proposed to prevent hemodynamic disturbances following long-term spaceflights, by means of producing a positive pressure gradient in the peripheral vascular system, which prevents deposition of blood in the lower half of the body. Various G-suit designs are used: elastic knit fabric, cuffs, etc. A G suit that exerts excess pressure on the lower limbs and abdominal wall causes decrease in capacity of the venous system of these regions and, consequently, prevents deposition of blood [9].

Suits with variable positive pressure, which control the increase in venous return of blood to the heart, depending on the condition of the cardiovascular system, elicit an active effect on peripheral hemodynamics. At the present time, devices with variable pressure are used primarily to prevent thrombus formation in veins of the lower limbs when patients are on bedrest for long periods of time [1, 4, 13-15]. In addition, various chambers have been designed which make use of this effect in cases of acute and chronic cardiovascular insufficiency [2, 11, 16]. However, concurrent elevation of pressure

over the entire area of chambers is not as effective as pressure of the "traveling wave" type (successive elevation of pressure in chamber sections from the periphery to the center). A device has been proposed in the form of a tubular bandage placed over the limb, the input end of which is connected to a pressure source and output, the atmosphere [3]. This device is intended for producing cyclic, evenly rising pressure on soft tissues of the limbs when it is necessary to maintain or restore blood pressure, for example during resuscitation procedures in terminal states when one observes deposition of blood in peripheral vessels of the limbs. A device was developed, in which production of positive pressure is synchronized with respiratory phases, to stimulate circulation in individuals whose breathing is controlled [7]. With some devices, it is possible to successively generate pressure of 60-100 mm Hg in cuffs at intervals of 15-60 s [1, 4]. However, production of pressure is not synchronized with cardiac function in any of these devices.

In developing a device for pneumatic massage of the extremities [5], the principle was applied of generating successively rising positive pressure, from the periphery to the center, on soft tissues of the extremities, which is synchronized with cardiac function. So-called "exogenous counterpulsation" (ECP) is obtained with this device, i.e., pressure to the peripheral vascular system in diastole. In other words, this device creates the effect of a "peripheral heart." Its advantages are that it is possible to control pressure in the cuffs as a function of condition of the cardiovascular system, the technique is simple and reliable. The same principle was applied in developing a device for ECP [6], the distinction of which is that there is a cuff for intensive action. A simplified version of the device for pneumatic massage of the limbs [8] is designed for hemodynamic ECP in emergency medicine.

In this study, we tested the effect of ECP on hemodynamics of the brain and lungs, as well as central hemodynamics at rest and during orthostatic tests in order to evaluate the use of this method in the early recovery period following simulated or real spaceflight.

#### Methods

A total of 20 healthy men, 25-35 years of age, participated in the tests. In the 1st series of studies, which were conducted in the morning, 1.5-2 h after breakfast with the subjects in recumbent position, we set pressures of 60 and 90 mm Hg in the cuffs. Cuff pressure (3 cuffs on the lower extremities and 2 on the upper) was produced in diastolic phase of the heart. Using a bipolar rheograph and Montedel recorder, we recorded the rhencephalogram (REG) in the frontomastoid (bilateral) and bimastoid leads.

In the 2d series, at the same time of day, we performed a passive orthostatic test, which consist of a 5-min period in orthostatic position at an angle of 70° without ECP and 5-min period with ECP. Cuff pressure was selected on an individual basis with the subjects in recumbent position. Absence of venous wave on the REG, elevation of diastolic index (DSI) on the REG of no more than 100% and absence of discomfort served as indicators of adequate ECP.

During the orthostatic tests, in addition to REG, we used a tetrapolar rheograph to record the rheogram (RG) of the lung and torso in order to determine stroke

(SV) and circulation (CV) volumes, and we also measured arterial pressure (BP) after Korotkov. Analysis of the REG and RG of the lung was performed by the method of Kh. Kh. Yarullin [10], systolic volume was calculated using the formula of Kubicek.

## Results and Discussion

Before the tests, at rest, pulsed delivery of blood, tonus and elasticity of cerebral vessels were within the normal range for the subjects' age.

When testing the effect of ECP on hemodynamics in recumbent position, we generated successively 60 and 90 mm Hg pressure in the cuffs. At 60 mm Hg, we observed changes in the same direction in pulsed delivery of blood to the three reservoirs of the brain examined, which increased by a mean of 10% in the hemispheres and 45.9% in the vertebrobasilar system, i.e., there was marked increase in pulsed delivery of blood, particularly in the vertebrobasilar system (Figure 1). This parameter increased even more with pressure of 90 mm Hg. Thus, pulsed delivery of blood increased by 16.6% in the hemispheres and 114% in the vertebrobasilar system, as compared to baseline data.

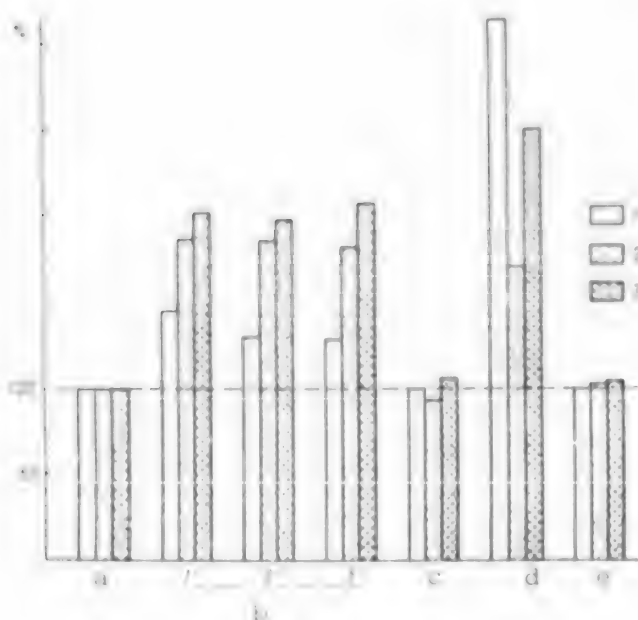


Figure 1.  
Changes in pulsed delivery of blood, tonus of arterioles and veins (%) under effect of various ECP modes with subjects in recumbent position

- a) baseline
- b,d) ECP 60 and 90 mm Hg, respectively
- c,e) corresponding recovery periods
- 1) pulsed delivery of blood
- 2) DSI
- 3) DCI [diacrotic index]

With ECP, the parameter of tonus of large-caliber arteries ( $\Delta/T$ ) rose by a mean of 16% in the hemispheres, and this increase in tonus was the same at pressures of 60 and 90 mm Hg. This parameter rose insignificantly in the vertebrobasilar system. The parameters of tonus of arterioles (DCI—diacrotic index), veins and venous efflux (DSI)\* of cerebral hemispheres showed virtually no change with ECP. In the vertebrobasilar system, these parameters increased significantly: DCI increased by 87.3% and DSI by 131.6% at pressure of 60 mm Hg and held at this level for the entire period of counterpulsation; at pressure of 90 mm Hg, DSI of the bimastoid REG reached +133.6% (see Figure 1).

In the orthostatic test without ECP, heart rate (HR) increased by a mean of 10/min, SV decreased by 30% and CV by 20%. Pulsed delivery of blood diminished by a mean of 66 and 30%

\*Since venous plethora and efflux from an organ are determined mainly by the condition of medium- and small-caliber veins [12], REG DSI reflects not only the state of venous efflux, but tonus of venules and veins [10].

in the hemispheres, by 31% in the vertebrobasilar system and by 44% in the lungs, in the presence of noticeable decline of parameters of tonus of arteries of large, medium and small calibers and veins (Figure 2, I-IV).

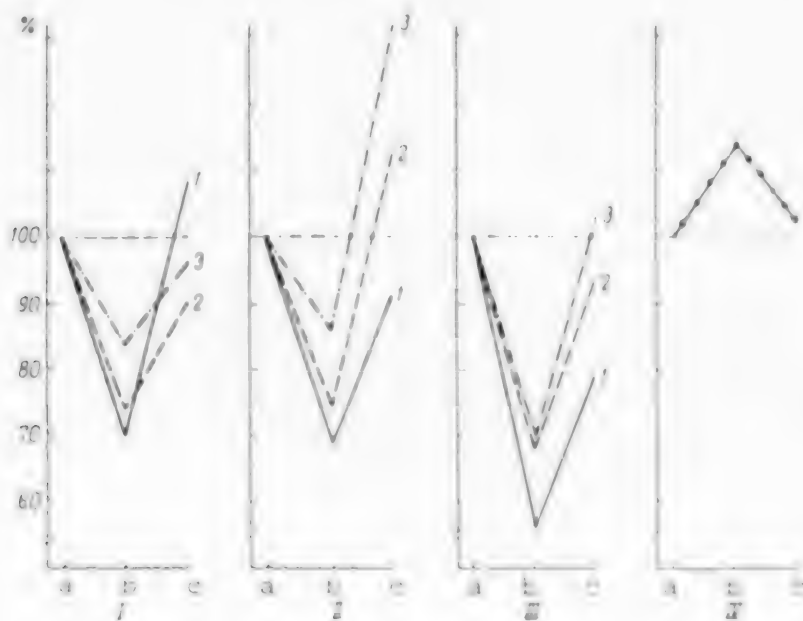


Figure 2. Changes in pulsed delivery of blood (1), tonus of arterioles (2) and veins (3) during passive orthostatic test without and with use of ECP

- |                       |                                 |
|-----------------------|---------------------------------|
| I) frontomastoid lead | a) recumbent position           |
| II) bimastoid lead    | b) orthostatic test without ECP |
| III) lung             | c) orthostatic test with ECP    |
| IV) HR                |                                 |

When the orthostatic test was combined with ECP, there was considerably less change in central and peripheral hemodynamics. Thus, pulsed delivery of blood to the hemispheres even increased by 19%, while the parameters for the vertebrobasilar system were only slightly below baseline levels. In the lung, the decrease in pulsed delivery of blood was less marked than during the test without ECP.

During the orthostatic test with ECP, the parameters of tonus of fine arteries and arterioles, venules and veins of the hemispheres and lung showed virtually no differences from their values in recumbent position: DCI exceeded baseline values by 13% in the vertebrobasilar system and DSI, by 31% (see Figure 2, II).

HR, BP, SV and CV changed less under the effect of ECP during the orthostatic test than in the test without ECP.

Thus, use of ECP with subjects in recumbent position led to increase in parameters of pulsed delivery of blood to the brain, more so in the vertebrobasilar system. The extent of elevation of this parameter depended on pressure in the



cuffs: at 90 mm Hg, pulsed delivery of blood to the brain changed more than at 60 mm Hg. The significant changes in tonus of arterioles and veins primarily in the vertebrobasilar system could be very important to supply of blood to the brain in situations that lead to ischemia. An increase in pulsed delivery of blood and parameters of tonus of cerebral vessels was noted throughout the period of using ECP.

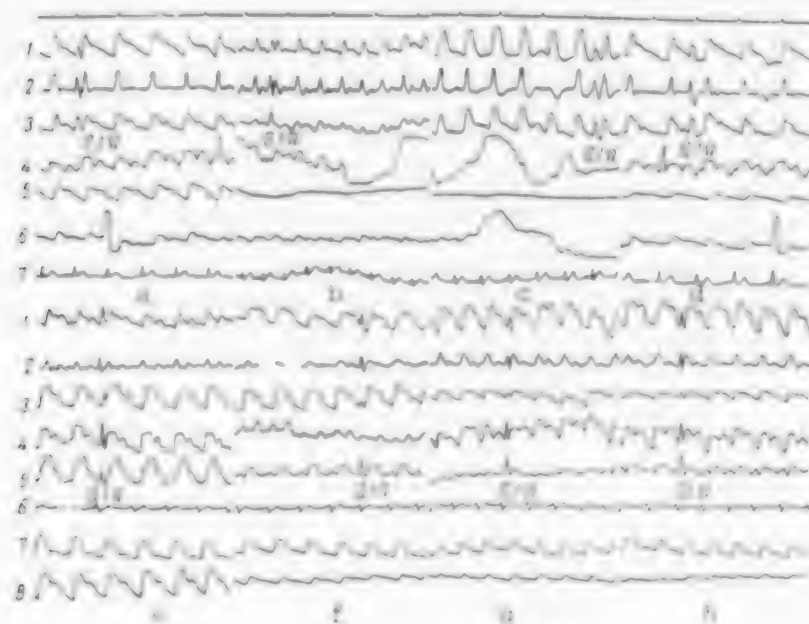


Figure 3. Regional RG of subject Sh. during orthostatic test without and with use of ECP

- 1) REG in frontomastoid lead, right
- 2) its first derivative
- 3) REG in frontomastoid lead, left
- 4) REG in bimastoid lead
- 5) RG of right lung
- 6) ECG in 2d standard lead
- 7) RG of torso
- 8) FPG [expansion unknown] of third finger
- a) baseline, subject in recumbent position
- b) 1st min of orthostatic test
- c) 4th min of orthostatic test, precollaptoid state
- d) recovery in recumbent position
- e) recumbent position + ECP
- f) 1st min of orthostatic test with ECP
- g) 5th min of orthostatic test with ECP
- h) recovery in recumbent position

The submitted data also indicated that ECP is an effective means of increasing blood supply to the brain and lungs by virtue of increasing SV, CV and BP, which diminish more or less in the presence of orthostatic factors. The

increase in delivery of blood to the regions in question and SV under the influence of ECP made it possible to increase CV at a lower HR, i.e., it caused more economical work of the heart. This helped retain hemodynamic homeostasis during orthostatic tests. It should be noted that, with use of ECP, all subjects reported improved wellbeing in orthostatic position. The sensation of blood rushing to the legs and discomfort that appeared in some subjects in erect position without ECP disappeared. Rhythmic compression of soft tissues of the limbs synchronized with the cardiac rhythm during the orthostatic test increased venous return of blood to the heart and maintained BP level, thus improving blood supply to the brain and heart. Ultimately, this enhanced orthostatic tolerance.

Use of ECP prevented development of syncope during the orthostatic test in 4 out of 6 subjects. Without ECP, all 6 subjects developed presyncopic and syncopic states during the orthostatic test performed after 49-day anti-orthostatic [head-down tilt] hypokinesia (Figure 3).

Use of ECP as a simple, reliable and nontraumatic method of improving blood supply to vital organs (particularly the brain) may be beneficial in the case of development of orthostatic hypotension.

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EFFECT OF OXYGEN INHALATION ON RESPIRATORY FUNCTION DURING EXERCISE AND EXPOSURE TO ADDED RESISTANCE TO RESPIRATION

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (manuscript received 22 Dec 86) pp 59-62

[Article by I. S. Breslav, G. G. Isayev and K. S. Rymzhanov]

[English abstract from source] Experiments were performed to investigate the effect of added resistance to inspiratory, expiratory and inspirator-expiratory respiration on lung ventilation and  $p_A CO_2$  during exercise when breathing air or oxygen. Increase of resistance to respiration, particularly inspiratory-expiratory, reduced the work-induced growth of ventilation which caused  $p_A CO_2$  to increase. Oxygen inhalation aggravated those shifts significantly. The adverse effect of hyperoxia on the respiration function when exercise was combined with added resistance to respiration seems to be associated with inhibition of the respiratory center produced by the lack of hyperoxid stimulation.

[Text] Breathing oxygen or hyperoxic gas mixtures attenuates changes related to metabolic acidosis, which develops during intensive exercise [1, 13, 16, 17]. These changes are aggravated when exercise is combined with a mechanical load on the respiratory system [3, 8]: under such conditions, respiratory acidosis may be added to metabolic acidosis and, in some cases, hypoxemia as well, and for this reason hyperoxic respiratory mixtures have been proposed for man when working in the presence of increased resistance to gas flow in the airways [6, 15, 21].

At the same time, such adverse reactions to hyperoxia as vascular spasm and hypoventilation are well-known; as a result of such responses delivery of oxygen to tissues may fail to improve and, on the contrary, worsen, not to mention the toxic effects of long-term exposure to high  $pO_2$  [17].

There may be prevalence of either positive or negative effects of hyperoxia under various conditions. Our objective here was to investigate the effect of pure oxygen inhalation on human respiratory function during prolonged intermittent exercise with added resistive (nonelastic) resistance to respiration. We selected pulmonary ventilation reactions and criteria of its

adequacy--CO<sub>2</sub> tension in alveolar gas, as the most important indicators of this effect.

## Methods

The studies were pursued on 4 healthy men 25 to 35 years of age.

Respiratory parameters were recorded using a spirographic unit developed in the laboratory [2]. The dynamics of inspiratory activity of the respiratory center were determined by a noninvasive method, according to maximum rate of elevation of inspiratory pressure in the airways at the start of inspiration ( $dp/dt_{\text{imax}}$ ), for which purpose we used a pressure transducer connected to the space underneath the mask, the signal from which was fed through a differentiating circuit to an automatic recorder. PCO<sub>2</sub> of alveolar gas ( $p_{\text{ACO}_2}$ ) was recorded on an MKh 6202 mass spectrometer, which was also used to determine gas exchange, for which purpose the sample of exhaled air was first passed through a mixer. Heart rate was determined by using an electrocardiograph.

The subjects exercised on a cycle ergometer at an intensity that was selected for each of them so as to reach minute volume of about 30 l, which corresponded to 60 to 100 W. After recording resting parameters for 10 min, we ran 7 5-min exercise periods ("heats"), alternating with 10-min rest periods, so that the entire test lasted about 2.5 h.

Resistive resistance to respiration (20 cm water · l<sup>-1</sup> · s)--inspiratory, expiratory or inspiratory-expiratory--was produced by means of a perforated diaphragm that was inserted into the appropriate channel of the respiratory circuit. Data obtained during free breathing (without resistance) served as a control.

Some of the tests were performed while breathing air and some with inhalation of pure oxygen. All test variants were alternated in random order.

The parameters recorded at rest and at the stable phase of exercise (5th min) with free breathing and different forms of resistance during inhalation of air and oxygen were compared by methods of variation statistics.

## Results and Discussion

At rest, added resistance to respiration did not elicit significant changes in parameters of respiratory function regardless of whether the subjects breathed air or oxygen.

However, during exercise these factors already had an appreciable effect on pulmonary ventilation (Figure 1).

The increase in ventilation elicited by exercise was usually less marked with added resistance to respiration than with the same exercise load and free breathing. The decline in ventilatory response to exercise was the most marked when inspiratory-expiratory resistance was used and least marked with expiratory resistance, when the difference from the control was found to be insignificant.



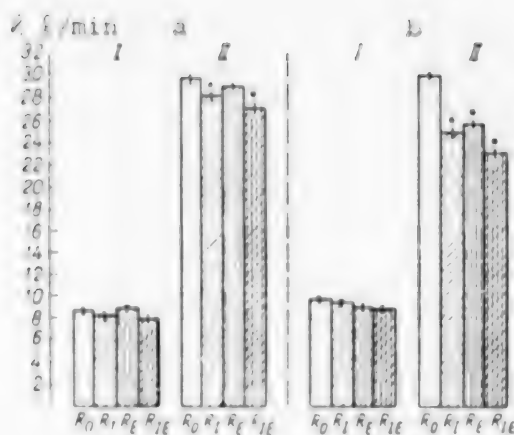


Figure 1.

Pulmonary ventilation  $\dot{V}$  (l/min) (M±m, n = 28) when breathing air (a) and oxygen (b) at rest (I) and during exercise (II)

Here and in Figure 2:

$R_0$ ) free breathing

$R_I$ ) inspiratory resistance

$R_E$ ) expiratory resistance

$R_{IE}$ ) inspiratory-expiratory,

\*Reliable differences ( $p < 0.05$ )

between data with  $R_I$ ,  $R_E$ ,  $R_{IE}$  and

$R_0$ , n = 28

Breathing oxygen had a noticeable aggravating effect on this hypoventilation change. As a result, the very mildest increase in ventilation was observed when exercise was performed under hyperoxic conditions with inspiratory-expiratory resistance.

As a result of poorer ventilation in response to exercise there was a rise in  $pCO_2$  of alveolar gas (Figure 2), which is inherent in the combination of exercise and increased resistance to respiration [3, 8]. True, when breathing air significant hypercapnia developed only with expiratory and, particular, inspiratory-expiratory resistance. But when the subjects breathed oxygen, the hypercapnic change was manifest with all types of resistance: least with inspiratory, most with inspiratory-expiratory.

Let us consider the dynamics of these parameters (see Figure 2). During all 7 "heats" pulmonary ventilation remained rather stable with inspiratory and expiratory resistance, and it diminished appreciably only with inspiratory-expiratory resistance. At the same time, hypercapnia

gradually increased with both air and oxygen and all forms of resistance.

When inhaling oxygen, alveolar  $pCO_2$  with inspiratory-expiratory resistance to respiration reached 66-68 mm Hg in the last "heats" for some subjects.

This change was unrelated to any changes in overall exchange of gases: the rise in oxygen uptake and  $CO_2$  output, which occurred during exercise, was about the same under all test conditions.

As we know, in the presence of resistive loads the respiration-controlling system does not remain passive. Efferent activity, the "motor output" of the respiratory center increases ([3] and others). This compensates, at least in part, added resistance to respiration. In our studies, such a compensatory reaction to resistance to respiration was manifested by elevation of the parameter that reflects so-called central inspiratory activity,  $dp/dt_{I_{max}}$ . During exercise with inspiratory-expiratory resistance this parameter showed more than 5-fold increase when breathing oxygen, as compared to exercise without resistance, but it increased only by 3 times under the same conditions when breathing oxygen ( $p < 0.01$ ).

The demonstrated depressive effect of hyperoxia on reactions of the respiratory system when exercise is combined with resistive resistance to respiration is no doubt attributable to exclusion of the hypoxic stimulus which is transmitted through chemoreceptors of the carotid body and plays some part in controlling respiration under normoxic conditions as well [5]. During exercise, the

significance of this stimulus increases considerably, as noted by some authors [14, 20] and shown by special studies conducted in our laboratory [9, 10].

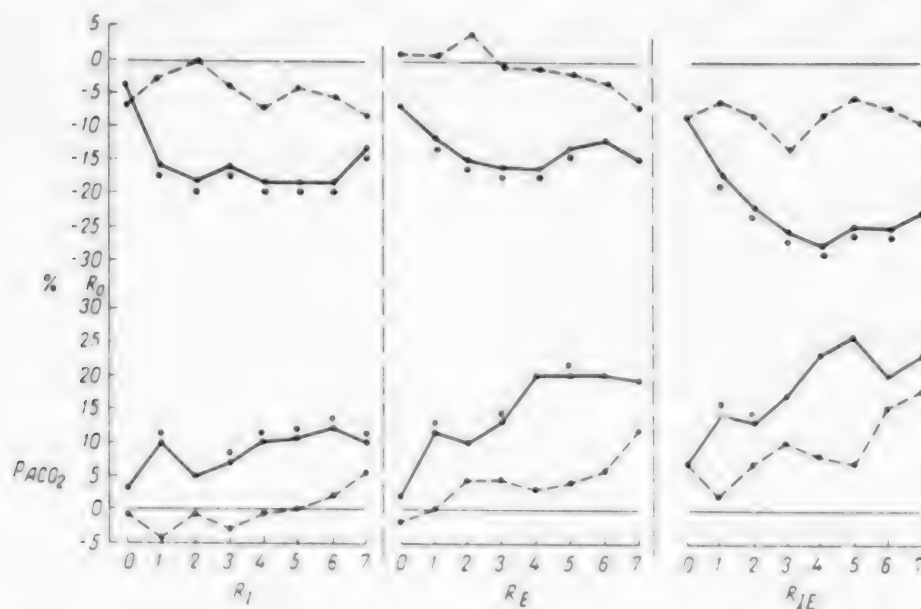


Figure 2. Effect of added resistance to respiration on dynamics of pulmonary ventilation ( $\dot{V}$ ) and alveolar  $p\text{CO}_2$  ( $p_{\text{ACO}_2}$ ) during exercise

X-axis: 0--rest, 1, 2, 3, 4, 5, 6, 7--sequential heat numbers; y-axis:  $\dot{V}$ ,  $p_{\text{ACO}_2}$ --deviations from values recorded with  $R_0$  (%); dash line--mean data when breathing air, boldface line--when breathing  $\text{O}_2$  ( $n=28$ ); asterisk indicates reliability of differences ( $p<0.05$ ) between data obtained with inhalation of  $\text{O}_2$  and air.

Recently, a report was published [18] to the effect that added resistance to respiration both at rest and particularly during exercise elicits reactions not only of the respiratory but cardiovascular system: heart rate, myocardial oxygen uptake and systolic blood pressure increase. According to our data, it can be assumed that hyperoxia attenuates these reactions also. Indeed, while the heart rate increased in the vast majority of cases, when exercising with inspiratory, expiratory and inspiratory-expiratory resistance while breathing air, as compared to exercise without resistance, in analogous tests where the subjects breathed oxygen an opposite reaction was observed in most cases.

Of course, these facts cannot be interpreted as evidence of the unequivocally adverse effect of elevated  $p\text{O}_2$  of the respiratory environment on human work capacity with added resistance to respiration. Under certain conditions, hyperoxia may also have a beneficial effect.

Thus, conditioned athletes who breathed a mixture with 35%  $\text{O}_2$  demonstrated higher values for the threshold of anaerobic metabolism, maximum oxygen uptake and tolerance time for near-maximum loads when submitted to a progressively increasing work load on a cycle ergometer with inspiratory resistance of 6.5 cm water  $\cdot \text{l}^{-1} \cdot \text{s}$  than in analogous experiments using air [15]. In man, signs of diaphragm fatigue, as recorded on the electromyogram, during breathing

with added inspiratory resistance appeared much later when he breathed oxygen, rather than air [19].

At the same time, for moderate but prolonged exercise with high resistance to respiration, particularly inspiratory-expiratory, inhalation of pure oxygen is a factor that worsens respiratory function, as demonstrated by the results of this study.

On the basis of the principle of minimization of respiratory and circulatory function, a mathematical model was developed with our participation [4], which permits deriving optimum concentrations of oxygen in the inhaled gas mixture for varying intensity of exercise. It can be predicted on the basis of this model that higher than nominal  $pO_2$  should cause other than optimum parameters of pulmonary ventilation and hemodynamics. Efforts have been made to use a strictly mathematical method to determine  $pO_2$  of the gas atmosphere that best corresponds to oxygenation of the body in order, for example, to validate recommended respiratory mixtures [6, 11].

We believe that expressly such a differentiated approach, which considers both metabolic requirements of the body and mechanical loads on the human ventilation system, will ultimately solve the problem of both permissible levels of respiratory resistance during exercise [12] and optimum  $pO_2$  levels in respiratory mixtures under such conditions.

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POLAR WORKER ADAPTABILITY TO ANTARCTIC HIGH ALTITUDES

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (manuscript received 18 Mar 87) pp 62-66

[Article by A. A. Aydaraliyev, A. L. Maksimov and T. B. Chernook]

[English abstract from source] The examinations were carried out during the 27th Soviet Antarctic expedition. Baseline data were collected before the departure of the test subjects to the Antarctic Region. Prior to their ascent to the high mountain area they were divided into two groups with a high and a low level of hypoxic tolerance in terms of the work capacity index calculated on the basis of standard bicycle ergometry tests. Heart rate, body temperature and salivary content of sodium and potassium were measured 6 times a day at 4-h intervals. The results obtained were treated by non-parametric tests. It was found that on adaptation day 30 the subjects with low hypoxic tolerance and nonspecific resistance developed changes in biorhythm amplitudes and phases and showed ultradian components with a 12-hour period. By contrast, the subjects with high hypoxic tolerance retained the ability to maintain circadian patterns. By the middle of the wintering time the circadian rhythms shifted towards ultradian components regardless of individual hypoxic tolerance.

[Text] When solving problems of medical support of long-term spaceflights, it is necessary to take into consideration adaptability of individuals differing in level of nonspecific resistance. Environmental conditions that man encounters at the intracontinental Vostok Station in Antarctica are similar to long-term spaceflight factors, from the standpoint of stress-producing elements for man. Marked hypoxia combined with extremely low temperatures and geomagnetic fluctuations, dry air, virtually sterile atmosphere, absence of customary alternation of day and night, the closed group and sensory deprivation is an incomplete list of factors the effects of which on man simulate in many respects the conditions that may be encountered during long-term spaceflights.

There are many works dealing with investigation of the function of different physiological systems under Antarctic conditions, in particular, there have



been many attempts at studying the effect of polar day and night on biorhythms of polar workers at both coastal and intracontinental stations [7-9, 31]. From the standpoint of assessing adaptation, the survey of polars at the Vostok Station is of some interest; it was conducted among all those spending the winter there and separately among groups as related to degree of tolerance to hypoxia. It is known that adaptation to an altitude of about 3500 m above sea level in the Pamirs and Tien Shan proceeds in several stages, and the stable phase of adaptation is formed essentially by the 45th day [15, 17, 31]. Considering the extreme conditions at the intracontinental Vostok Station, it can be assumed that considerably more time is required to reach an adapted state, and the responses will be different for individuals with different levels of nonspecific resistance.

At the present time, evaluation of functional state of the entire body exposed to stress factors, as well as degree of adaptation to extreme environmental conditions, is made by different methods, ranging from fine biochemical analyses to construction of mathematical models. In particular, biorhythmological criteria have been used extensively in recent times to study the physiological mechanisms of adaptation [1, 3, 4, 12, 33, 16, 18, 19, 22, 23, 26-29]. However, in order to assess the functional state of an organism it is important to know not only the absolute values of different parameters and their circadian periodicity, but reciprocal correlation, which reflects mobilization of different systems in the integral response to extreme environmental factors [24].

We have made an attempt here to assess the adaptability of individuals differing in level of tolerance to hypoxia on the basis of investigation of the structure of biorhythms and correlations between different parameters.

#### Methods

This study was pursued at the Vostok Station (3485 m above sea level) during the 27th Soviet expedition to Antarctica (17 people). The subjects were taken to the coast of Antarctica on a ship, then flown to the intracontinental Vostok Station. Data obtained when the subjects were examined prior to being taken to Antarctica (14 people) served as the baseline. Parameters were recorded at 0700, 1100, 1500, 1900 and 0300 hours local time. We selected the simplest and most informative methodological procedures, which enabled us to assess functional state when working under the conditions of antarctic wintering: temperature, pulse, potassium and sodium levels in saliva. The latter, in particular, reflects activity of the adrenosympathetic system. Sublingual temperature was measured with a TFD-1 sensor, pulse rate was counted on the cardiogram, sodium and potassium levels in saliva samples were analyzed by flame photometry using a Zeiss photometer [25]. The data were processed using computer analysis program [5, 18, 11, 30] and nonparametric statistic method (rank correlation after Spearman) with the adopted level of reliability,  $p < 0.05$ . Cluster analysis, in spite of its circumstantial nature, is a convenient model for assessment of group biorhythmological status and it permits determination of parameters of rhythmic components on the basis of an approximated hypothetical curve. On this basis one can judge the extent of tension with which a given system functions. The arbitrary separation into groups with high and low level of hypoxia tolerance was made by the method of H. M. Bryukhov

and A. A. Aydaraliyev [20]. In accordance with this method, subjects are divided according to tolerance to exercise. The work capacity index thus obtained is correlated with resistance to hypoxia with a high degree of reliability ( $r = +0.92$ ). This thesis had been tested for several years in high-altitude mountain regions. Assessment of resistance to hypoxia and its prediction in polar workers were made in baseline studies before they ascended to the high-altitude region of Antarctica.

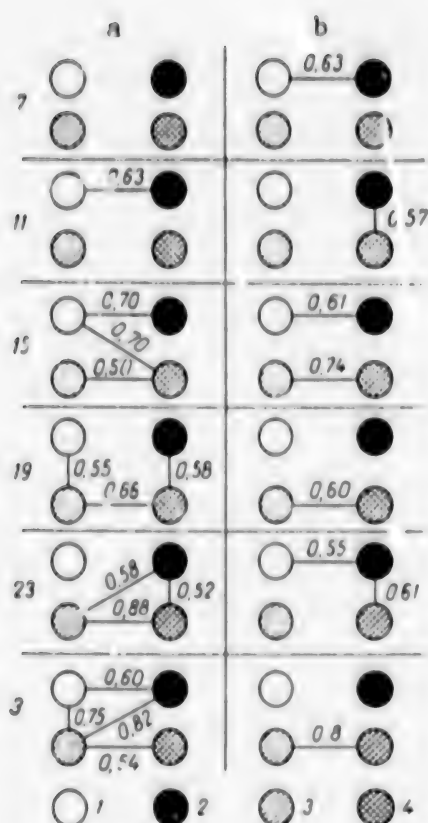
### Results and Discussion

The data obtained for the two arbitrary groups with high (1st group) and low (2d group) levels of resistance to hypoxia revealed that a circadian rhythm was retained in subjects of the 1st group on the 30th day of adaptation to alpine Antarctica according to the temperature parameter, whereas ultradian components with a period of 12 h appeared in the 2d group of subjects. There was shift to 9.9 h in the acrophase (versus 19.8 h in the 1st group). The latter group was characterized by a wide spread of amplitudes, which is indicative of greater adaptability of the subjects. The shift of the acrophase to the morning hours and (which is more important) appearance of ultradian rhythms are indicative of the relatively low adaptability of the 2d group. After 6 months of adaptation, no differences were demonstrable in biorhythmological status of the hypoxia-resistant and sensitive groups, which is indicative of the general equalizing effect of natural extreme factors and the same strategy of adaptation to them. At the same time, for the group as a whole, toward the middle of the wintering period circadian organization underwent significant alteration and differed considerably from the baseline findings and those made on the 30th day of adaptation.

Thus, it was established that there was a shift of circadian rhythms in the direction of ultradian components toward the middle of the wintering period according to all parameters examined. Autospectral analysis of pulse rate revealed that a significant part of the overall scatter of the process is concentrated at ultradian frequencies, and with a statistically significant 12-h rhythm there is change in mean level and phase of fluctuations referable to nocturnal hours (1.7 h). The change from a 24-h rhythm to a 12-h one and acrophase shift from daytime to night are indicative of significant desynchronization between levels of cardiac activity and customary activity of the body.

Investigation of biorhythms of excretion of sodium in saliva during polar night revealed increase in scatter of parameters in the group and their heterogeneity, as compared to baseline values. Only a 12-h rhythm was demonstrable, whereas there was a 24-h rhythm with 12-h component in the baseline studies. The acrophase was referable to 2.6 h, which is 1.7 h less than in the control studies. The 24-h rhythm of potassium excretion in saliva also changed to a 12-h rhythm with amplitude of fluctuations of 5.57 meq/l and acrophase at 2.5 h. S. I. Stepanova [29] believes that the mechanism of increase in amplitude of baseline fluctuations depends on activation of expenditure and restoration of energy and plastic resources of functional systems of the body.

As a rule, the circadian physiological rhythms are synchronized by geophysical and social sensors with a 24-h period and stationary amplitude-phase structure.



Correlation pleiads reflecting relationship between pulse, temperature and sodium and potassium levels in saliva of polar workers at Vostok Station in the middle of the wintering period

a) baseline studies

b) studies in middle of wintering period

7, 11, 15, 19, 23, 3) time of day

1) pulse

2) temperature

3,4) saliva sodium and potassium, respectively

Numerals within pleiads are the coefficients of correlation.

complete isolation). In spite of this, we observed a significant shift of temperature rhythm in the group of hypoxia-resistant subjects on the 30th day of adaptation, and no statistically significant 24- and 12-h rhythms were recorded in the middle of the wintering period in the group as a whole. Thus, retention of circadian rhythms under extreme conditions depends largely on both the level of nonspecific resistance of subjects and duration of exposure to extreme factors. Consequently, biorhythmological parameters can be used as an integral prognostic criterion of adaptation.

When isolated from external "time-setters" the subjects have mainly free-flowing rhythms. Capturing the rhythm by a compulsory, in particular social, clock leads to desynchronization of rhythms, and the circadian rhythms of different functions have different widths for the "capture window," for example, the temperature rhythm is the first to escape the control of the compulsory clock [4]. In this case, circadian rhythms of physiological functions undergo profound change, leading to chronophysiological adaptation of man to a new environment and change in amplitude and phase structure of circadian rhythms. In our opinion, this is expressly what happens in the middle of the wintering period, which is referable to polar night in Antarctica. The daily scatter of temperatures in the tested period became much narrower ( $0.3^{\circ}\text{C}$  versus  $0.8^{\circ}\text{C}$  in the baseline period), while the previous 24-h rhythm became statistically irrelevant. In the opinion of N. I. Moiseyeva [22], in essentially healthy people who are well-adapted to change in environmental conditions there is a wide scatter of values for the variable during a 24-h period and relatively stable structure of curves. In individuals who are poorly adapted, the curves reflecting dynamics of the same function differ considerably. They show less variation of values within a 24-h period. Consequently, the temperature parameter in our studies is indicative of significant tension of adaptation mechanisms in polar workers in the middle of the wintering period.

It is believed by some researchers [2] that the temperature rhythm is the most stable in both a free-flowing state and under the influence of certain factors (heavy physical labor, bedrest, complete isolation).

The fact that there is considerable tension in the middle of the wintering period of physiological functions, regardless of degree of resistance to hypoxia, is confirmed by analysis of correlation functions. The latter revealed that the correlations between systems vary at different times of day, i.e., the extent of participation of functional systems in integral activity of the body is not the same. On the whole, in the baseline studies (see Figure), positive correlations were found, starting in the afternoon (1500 h), for all tested parameters, although they were related not only through direct, but indirect connections ( $r = +0.5-0.88$ ) in the correlation pleiad. Toward the middle of the wintering period, not only was there change in structure, but reduction in correlations, i.e., their total number changed due to disjunction (1-2 parameters remained related). Since there is a decrease in total coefficients of correlation, one can refer, on the whole, to reduction in closeness of correlations. All this is indicative of significant asthenization, which develops in polar workers at this stage of their stay.

The results of our studies have much in common with the data of other researchers. Thus, S. G. Krivoshekov [14] showed that an increase in correlation between sodium content of saliva and heart rate at rest is associated with increase in parameters of physical work capacity, while the period of human acclimation is associated, to some degree, with diminished synchronization of relations between these parameters. T. D. Semenova [26] established that there is a less close correlation between pulse, temperature and saliva sodium content in a subject during long-term hypokinesia (mean coefficient of correlation dropped significantly by the 90th day).

The decrease in both force and number of correlations observed by us in polar workers is indicative of desynchronization of functions of different elements of the biosystem and development of fatigue, which is typical of unsatisfactory adaptation. The body strives to adapt to conditions that are extreme for it by altering functional activity of different systems and corresponding strain on regulatory mechanisms (increase in cost of adaptation) [6].

Thus, on the basis of analysis of data referable to the middle of the wintering period, we arrived at the conclusion that polar workers do not demonstrate total adaptation at that time.

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EFFECT OF EXTERNAL ELECTROSTATIC FIELD ON CATECHOLAMINE SECRETION BY  
RAT ADRENALS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21,  
No 6, Nov-Dec 87 (manuscript received 20 Jan 87) pp 67-70

[Article by G. G. Artsruni, A. V. Zilfyan, N. R. Azgaldyan and R. A. Dovlatyan]

[English abstract from source] Experimental rats were exposed to an electrostatic field of 200 kV/m for 1, 24 or 6 hours a day for 6 days. The effect of this exposure on the structure and function of adrenal chromaffin bodies and the concentration of catecholamines and their precursors in blood and adrenals was investigated. Using histochemical fluorescent-microscopic and spectrofluorimetric methods, it was found that the exposure may modify the structure and function of adrenal chromaffins and the concentration of catecholamines in blood and adrenals. These changes depended on the exposure time. An hour exposure activated the secretory apparatus of adrenal chromaffins and enhanced catecholamine secretion to blood. A 24-hour exposure led to an increase in the concentration of catecholamines in adrenals and blood and a change in the organ cytoangioarchitectonics. A 6-day fractionated exposure resulted in a decrease of catecholamines both in blood and adrenals.

[Text] When aircraft fly below thunder clouds, the crews are exposed to external electrostatic fields (EEF) that are many times stronger than the natural background [1, 8].

Existing data concerning the effect of EEF on biological systems convince us of their high biological activity [6, 10]. We did not encounter any works dealing with the effect of EEF on secretion and levels of biogenous amines, which play an important part in maintaining homeostasis and development of adaptive reactions. We shall discuss here the effects of EEF on structural and functional state of adrenal chromaffin tissue and catecholamine content.

#### Methods

Experiments were performed on 86 mongrel, white male rats weighing 0.12-0.15 kg. EEF of 200 kV/m was produced by means of a unit of the condenser type with controllable field parameters [2]. We tested the effects of 1, 24 and

fractionated (6 h/day for 6 days) exposure. Immediately after exposure to EEf the animals were sacrificed by decapitation. Experiments were performed at the same time of day. In the 1st group of animals, we measured blood epinephrine and norepinephrine by the method of A. A. Shatalova [9], adrenal epinephrine, norepinephrine, dopa and dopamine by the method of E. I. Matlina [5]. All spectrofluorimetric readings were taken using an MF-4 Hitachi spectrofluorimeter. In the 2d group of animals, the adrenals were fixed in Carnoy fluid, paraffin sections were stained by conventional histological techniques. In order to demonstrate the nature and distinctions of topical distribution of catecholamines, we prepared fresh-frozen cryostat sections of the adrenals, which were treated by the method of Falk in the modification of Ye. M. Krokhina [4]. Fluorescence of catecholamines was measured using a method we had proposed previously [3].

The obtained data were submitted to statistical processing using the reliability criterion of Fisher-Student.

### Results and Discussion

After 1-h exposure to EEf, there was focal dissociation of the secretory system of adrenal chromaffin tissue. There was prevalence in the parenchyma of this gland of cells with markedly acidophilic compact cytoplasm, which was rich in RNA and proteins, with a relatively small basophil nucleus--functionally active dark cells (Figure 1a). As a rule the areas of dissociation were represented by cells with dramatically vacuolized cytoplasm and large, chromatin-poor nucleus (light cells). Fluorescence microscopy for quantitative analysis of catecholamines revealed that there was an appreciable decrease in their levels in the cytoplasm of secretory cells. These findings are consistent with biochemical parameters of norepinephrine and epinephrine content of the organ. There was an increased amount of catecholamines in blood (see Table). In chromaffin tissue, disturbances in the microcirculatory system were prominent, as manifested by dramatic dilatation of sinuses, stasis and plethora of the organ (Figure 1b). The walls of sinuses and adjacent tissues are saturated with plasma proteins. The vascular lumen shows many formed elements. Secretory cells in the immediate vicinity of sinuses are flattened and have signs of dystrophy. Treatment of adrenals with paraformaldehyde made it possible to detect isolated sites of fluorescence, mainly in cells in the immediate vicinity of sinuses (Figure 2a, b), in the presence of diffuse decrease in catecholamines, for this reason, parameters of fluorescence of catecholamines in the adrenals did not differ from the control level after such exposure, there being reliable decrease in epinephrine and norepinephrine content as demonstrated by a biochemical method. Blood showed virtually 2-fold elevation of epinephrine and norepinephrine levels (see Table).

In the case of exposure for 6 h/day for 6 days, the structure of the adrenal medulla was impaired, and there was extensive dissociation of the glandular system. Secretory cells were represented mainly by the light forms with very marked vacuolization of cytoplasm and signs of nuclear polymorphism. We observed formation of cavities lined with single and double columnar epithelium containing structureless eosinophilic material (Figure 1b). A noticeable decrease in specific fluorescence was found in the cytoplasm of secretory cells (Figure 2b). The structural and metabolic changes in adrenal tissue

under the effect of fractionated exposure were associated with decrease in epinephrine and norepinephrine content of both blood and the organ of experimental animals.

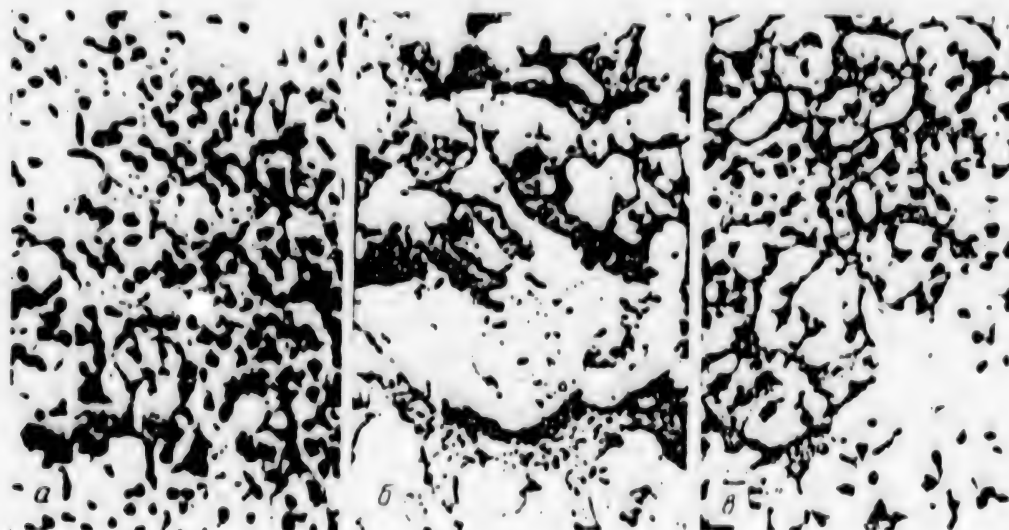


Figure 1. Adrenal chromaffin tissue of rats exposed to EEF; hematoxylin and eosin stain; lens 20 $\times$ , eyepiece 10 $\times$

- a) sites of dissociation and moderate stromal edema, prevalence of dark cells after 1-h exposure
- b) dilatation of sinuses, saturation of vascular walls with plasma proteins after 24-h exposure
- c) early stages of formation of retention cysts after fractionated exposure

Catecholamine content after exposure to EEF of 200 kV/m

Exposure time	Material	Epinephr., $\mu\text{g/g}$ or $\mu\text{g/g}\cdot\text{l}$	NE, $\mu\text{g/g}$ or $\mu\text{g/g}\cdot\text{l}$	Dopa, $\mu\text{g/g}$	Dopamine $\mu\text{g/g}$	Fluoresc. of NE-con taining cells, AU
0 (control)	Adrenals	$402.5 \pm 13.13$ (n 6)	$129.31 \pm 5.83$ (n 6)	$18.01 \pm 0.65$ (n 6)	$53.65 \pm 1.21$ (n 6)	$21.64 \pm 0.13$ (n 6)
	Blood	$4.85 \pm 0.093$ (n 10)	$1.36 \pm 0.102$ (n 10)			
1 h	Adrenals	$398 \pm 17.17$ (n 6)	$107.42 \pm 2.57^*$ (n 6)	$16.89 \pm 0.35$ (n 6)	$60.07 \pm 0.73^*$ (n 6)	$11.89 \pm 0.12^*$ (n 6)
	Blood	$5.87 \pm 0.12^*$ (n 9)	$1.91 \pm 0.093^*$ (n 9)			
24 h	Adrenals	$357 \pm 5.99^*$ (n 6)	$75.8 \pm 2.5^*$ (n 8)	$13.33 \pm 0.26^*$ (n 6)	$54.3 \pm 1.2$ (n 6)	$19.1 \pm 0.13^*$ (n 6)
	Blood	$10.78 \pm 0.61^*$ (n 8)	$5.11 \pm 0.221^*$ (n 8)			
6 h/day, 6 days	Adrenals	$308.66 \pm 5.0$ (n 6)	$123.81 \pm 4.2$ (n 6)	$14.55 \pm 0.27^*$ (n 6)	$55.22 \pm 1.15$ (n 6)	$17.36 \pm 0.12^*$ (n 6)
	Blood	$1.211 \pm 0.167^*$ (n 7)	$0.89 \pm 0.12^*$ (n 7)			

\*  $p < 0.05$

NE) norepinephrine

AU) arbitrary units

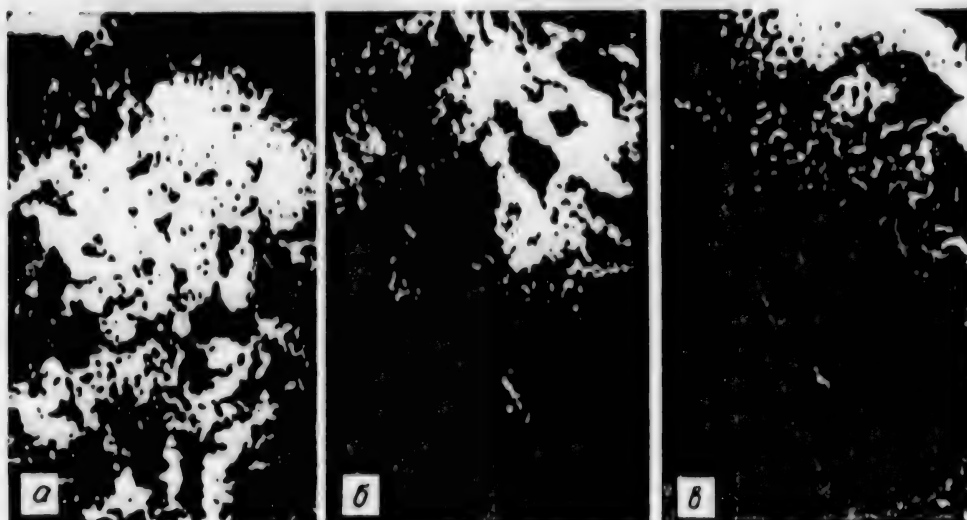


Figure 2. Distribution of catecholamines in chromaffin tissue of adrenals of control and experimental animals exposed to EEF; paraformaldehyde treatment

- a) specific fluorescence of catecholamines in secretory cell cytoplasm of control rats; lens 20 $\times$ , eyepiece 10 $\times$
- b) mild focal fluorescence of catecholamines of secretory cells after 24-h exposure; lens 20 $\times$ , eyepiece 7 $\times$
- c) decrease in specific fluorescence of secretory cell cytoplasm after 6-day exposure; lens 20 $\times$ , eyepiece 10 $\times$

In the course of the experiment, precursors of biogenous amines--dopa and dopamine-- in adrenal tissue were measured. Dopa content was diminished with 24-h and 6-day exposure, while dopamine was elevated only after 1-h exposure. This dependence of catecholamine precursor levels on duration of exposure is perhaps due to the inadequate effect of EEF on enzymatic decarboxylase and  $\beta$ -hydroxylase systems responsible for catecholamine synthesis.

Our studies revealed that exposure to EEF of 200 kV/m leads to impairment of structure and function of adrenal medulla, changes in levels of catecholamines and their precursors in the adrenals and blood, the severity and nature of observed changes being a function of duration of exposure. One-hour exposure to EEF was characterized by activation of the secretory system of adrenal chromaffin tissue and associated with more intensive passage of catecholamines into blood. These changes should be interpreted as one of the manifestations of adaptive responses of the adrenals to the physical factor we tested.

The high catecholamine levels in the adrenals and blood, in the presence of impaired cytoangioarchitectonics of the organ following 24-h exposure are indicative of functional stress on the secretory system of the adrenal medulla.

Exposure to EEF for 6 days led to decline of blood and adrenal catecholamine levels, which is indicative of decline in fractionated activity of adrenal chromaffin tissue.



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## TWO-DIMENSIONAL STATIC OTOLITH MODEL

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[Article by A. V. Kondrachuk and S. P. Sirenko]

[English abstract from source] A model of the otolith as a system with distributed parameters in which the otolith is described by a two-dimensional elastic plate fixed at its sides is presented. The behavior of the model in response to centrifugal and gravitational forces is discussed. Comparison of this model with experimental data yields the value of Young's module of the otolith membrane equal to  $10^2$ - $10^3$  dyne/cm<sup>2</sup>. It is shown that deformations of different compartments of the otolith are dissimilar even for a homogeneous otolith membrane, depending on its configuration and orientation relative to the force vector. It is concluded that intralabyrinthine pressure may be the cause of original nonhomogeneous deformation of hairs in receptor cells at rest.

[Text] There has been increased interest in the study of biophysical processes in the vestibular system because of development and use of new transport (space and aircraft). Mathematical models of behavior of different parts of the vestibular system, in particular, the otolith system which reacts to linear accelerations, play an important role here.

The otolith is an elastic plate about  $10^{-2}$  cm<sup>2</sup> in area and about  $10^{-2}$ - $10^{-3}$  cm thick [5, 10]. One can differentiate three elements of the otolith: 1) otolith membrane (OM) with otoconia (calcite crystals with 2-3 g/cm<sup>3</sup> density) immersed in a gelatinous substance; 2) macula--the aggregate of supporting and receptor hair cells; 3) submembrane space--intermediate layer between the OM and macula (Figure 1) [5, 12]. Each receptor hair cell has up to 100 stereocilia and one kinocilium, the deflection of the hair in the direction from the stereocilia to the kinocilium increases the frequency of spontaneous impulsation (FSI) of the corresponding nerve fiber and deflection in the opposite direction reduces FSI [4, 5, 7]. The receptor cells are specifically distributed over the macula, which leads to presence of regions with specific morphological polarization (Figure 2) [5, 6].

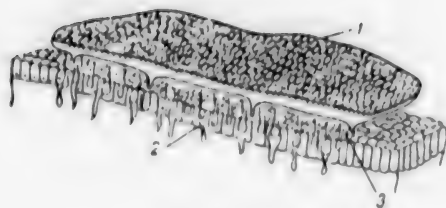


Figure 1.  
Otolith structure [12]  
1) OM                      3) submembrane  
2) macula                space

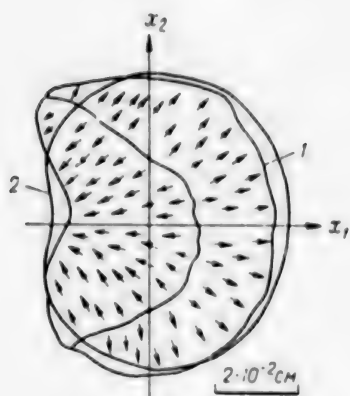


Figure 2.  
Morphological polarization of otolith [6]

- 1) outline of actual OM
- 2) outline of OM constructed using formula (17)

The otolith system functions on the following principle: as a result of density difference between the endolymph and OM, the acting force elicits an inertial shift (deformation) of the OM in relation to the macula, which leads to deflection of receptor cell hairs and, accordingly, to change in frequency of vestibular nerve impulsation. It is believed that a force parallel to the macular plane is effective [4, 5]. Many features of the otoliths are still unknown, for example: Is there deformation of the OM or does it shift as a whole under the effect of inertial forces? How is the OM attached on the macula and what are the mechanical parameters of the OM (Young's modulus, Poisson's coefficient, etc.)? Models that describe the otolith system as a mechanical system can be arbitrarily classified in the following three types: 1) system with distributed parameters (SDP), 2) system with concentrated parameters [14], 3) intermediate type of OM model [10]. The first type, which changes to models of the second and third type with maximum values of OM parameters, corresponds best to the actual physical structure and functional distinctions of otoliths.

At the present time, there are only a few works dealing with models of OM as an SDP [2, 11], due to the complicated mathematical description and absence of the necessary quantitative characteristics of physicomachanical parameters of OM.

W. J. Hudetz [11] was the first to try to consider OM as an SDP subject to static inertial forces in approximation of a general flat tense state. However, there were flaws in his study: the OM shape was represented very approximately; the equations that were solved mathematically were not inscribed in the system of coordinates to which the author turned by means of conform transformation for convenience in using a method of finite differences, which led to the wrong quantitative results.

We propose here a mathematical model of OM in the form of a two-dimensional elastic plate with secured edges, similar in shape to a real one. We examined the behavior of the model when OM is exposed to centrifugal and gravity forces.

#### Mathematical Model of OM

Let us examine the static behavior of the endolymph-OM system exposed to gravity and centrifugal forces:

$$\vec{f}^{(1)} = \rho_1 \vec{g} - \rho_1 [\vec{\omega} \cdot [\vec{\omega} \cdot (\vec{R} + \vec{r})]] \quad (1)$$

where  $\vec{\omega}$  is vector of angular velocity,  $\vec{g}$  is vector of gravity,  $\vec{R}$  is radius-vector, the modulus of which equals the distance from axis of rotation to start of coordinates of endolymph-OM system,  $\vec{r}$  is radius vector of arbitrary point in system,  $\rho_1$  and  $\rho_2$  are density of endolymph and OM, respectively.

Since  $|\vec{R}| \gg |\vec{r}|$  under real conditions, let us consider that force is distributed homogeneously, i.e.,

$$\vec{f}^{(1)} = \rho_1 \vec{g} - \rho_1 [\vec{\omega} \cdot [\vec{\omega} \cdot \vec{R}]] \quad (2)$$

Let us note that to meet static requirements vectors  $\vec{\omega}$  and  $\vec{g}$  are parallel, and we shall describe their projections as follows:

$$\begin{aligned} g_1 &= g \sin \alpha \cos \beta, \quad g_2 = g \sin \alpha \sin \beta, \\ g_3 &= g \cos \alpha, \\ \omega_1 &= \pm \omega \sin \alpha \cos \beta, \quad \omega_2 = \pm \omega \sin \alpha \sin \beta, \\ \omega_3 &= \pm \omega \cos \alpha, \end{aligned}$$

where  $\alpha$  is the angle between axis  $x_3$  and vector  $\vec{g}$ ,  $\beta$  is the angle between axis  $x_1$  and projection of vector  $\vec{g}$  on plane  $x_1x_2$  (+ and - signs are selected in accordance with direction of vector  $\vec{\omega}$ ).

There will be change in endolymph pressure  $P$  under the influence of force (2), and according to the hydrostatics equation,

$$\text{grad } P = \vec{f}^{(1)},$$

$$P = p_0 + \rho_1 \sum_{j=1}^3 a_j x_j \quad (3)$$

where  $a_j = g_j - [\vec{\omega} \cdot [\vec{\omega} \cdot \vec{R}]]$ ,  $p_0$  is endolymph pressure in the absence of force (2).

Let us turn to derivation of the equation that defines the vector field of OM displacement when in the field of action of encolymphatic pressure (3) and force (2) approximated to a generalized flat tense state. We shall proceed from general statistical equations of elasticity theory:

$$\sum_{j=1}^3 \frac{\partial \tau_{ij}}{\partial x_j} + \rho_2 a_i = 0 \quad (i = 1, 2, 3), \quad (4)$$

where  $\tau_{ij}$  are components of stress tensor.

On the basis of OM we shall assume that  $\tau_{31} = \tau_{32} = 0$ ,  $\tau_{33} = -P$  ( $x_3 = \pm h/2$ ,  $h$  is OM thickness).

Let us consider the third equation of system (4):

$$\sum_{j=1}^3 \frac{\partial \tau_{3j}}{\partial x_j} + \rho_2 a_3 = 0. \quad (5)$$

Let us describe  $\tau_{33}$  in the following form:

$$\tau_{33} = -\rho_1 \sum_{j=1}^2 a_j x_j - \rho_2 a_3 x_3 + \tau_{33}^*. \quad (6)$$

Substituting (6) in (5), we shall obtain

$$\sum_{j=1}^2 \frac{\partial \tau_{3j}}{\partial x_j} + \frac{\partial \tau_{33}^*}{\partial x_3} = 0. \quad (7)$$

The boundary conditions for  $\tau_{33}^*$  have the following appearance:

$$\tau_{33}^*|_{x_3 = \pm h/2} = -\rho_0 + (\rho_2 - \rho_1) a_3 x_3. \quad (8)$$

Using experimental data:  $p_0 \sim 10^6$  dyne/cm<sup>2</sup> [1],  $h \sim 10^{-3}$  cm,  $(\rho_2 - \rho_1) \lesssim 1$  g/cm<sup>3</sup> [5, 10], we shall obtain inequality

$$\rho_0 \gg |(\rho_2 - \rho_1) a_3 \frac{h}{2}|.$$

This inequality occurs with a wide range of changes in  $a_3$  which overlaps physiologically permissible values. For this reason, with  $x_3 = \pm h/2$  we can assume that  $\tau_{33}^* \approx -P_0$ .

By equating in equation (7)  $x_3 = \pm h/2$  and considering that  $\tau_{31}$  and  $\tau_{32}$  equal zero on the basis of OM, we shall obtain  $\frac{\partial \tau_{33}}{\partial x_3} = 0$ , hence,  $\tau_{33}^* \approx -P$  over the entire thickness of OM.

Inserting  $\tau_{33}$  in the equation:

$$\tau_{33} = \frac{E}{(1+\nu)(1-2\nu)} \times \\ \times \left[ (1-2\nu) \frac{\partial U_3}{\partial x_3} + \nu \operatorname{div} \vec{U} \right],$$

where  $E$  is Young's modulus,  $\nu$  is Poisson's coefficient and  $U_i$  ( $i=1, 2, 3$ ) are components of displacement, and disregarding terms of the order of  $h$ , we shall obtain:



$$\frac{\partial U_1}{\partial x_2} = \frac{(1+v)(1-2v)}{E(1-v)} \times \left[ p_0 + p_1 \times \right. \\ \left. \times \sum_{j=1}^2 a_j x_j \right] - \frac{v}{1-v} \left( \frac{\partial U_1}{\partial x_1} + \frac{\partial U_2}{\partial x_2} \right).$$

Inserting this expression in the equation:

$$\tau_{11} = \frac{E}{(1+v)(1-2v)} \left[ (1-2v) \times \right. \\ \left. \times \frac{\partial U_1}{\partial x_1} + v \operatorname{div} \vec{U} \right], \\ \tau_{22} = \frac{E}{(1+v)(1-2v)} \left[ (1-2v) \times \right. \\ \left. \times \frac{\partial U_2}{\partial x_2} + v \operatorname{div} \vec{U} \right],$$

we shall have:

$$\tau_{11} = \frac{v}{1-v} \left[ p_0 + p_1 \sum_{j=1}^2 a_j x_j \right] + \frac{vE}{1-v^2} \times \\ \left[ \frac{\partial U_1}{\partial x_1} + \frac{\partial U_2}{\partial x_2} \right] + \frac{E}{1+v} \frac{\partial U_1}{\partial x_1}, \quad (9)$$

$$\tau_{22} = \frac{v}{1-v} \left[ p_0 + p_1 \sum_{j=1}^2 a_j x_j \right] + \frac{vE}{1-v^2} \times \\ \times \left[ \frac{\partial U_1}{\partial x_1} + \frac{\partial U_2}{\partial x_2} \right] + \frac{E}{1+v} \frac{\partial U_2}{\partial x_2}. \quad (10)$$

Substituting (9), (10) and the following equation

$$\tau_{11} = \frac{E}{2(1+v)} \left[ \frac{\partial U_2}{\partial x_1} + \frac{\partial U_1}{\partial x_2} \right]$$

in equation (4) and turning to averaged OM thickness values, we shall obtain:

$$\operatorname{grad} \operatorname{div} \vec{u} - \frac{1-v}{2} \operatorname{rot} \operatorname{rot} \vec{u} + \frac{1-v^2}{E} p_0 = 0, \quad (11)$$

where

$$\vec{u} = u_1 \vec{i} + u_2 \vec{j}, \quad \vec{a} = a_1 \vec{i} + a_2 \vec{j}, \quad p = p_0 + p_1 \frac{v}{1-v},$$

$$u_j = \frac{1}{h} \int_{-h/2}^{h/2} U_j dx_3.$$

We know [3] that the solution for equation (11) can be expressed using analytical functions  $\varphi(\xi)$  and  $\psi(\xi)$ :

$$\begin{aligned} \frac{E}{1+\nu} [u_1 + iu_2] &= V\omega^2(\xi) + \kappa\varphi(\xi) \\ &= \frac{\omega(\xi)}{\omega'(\xi)} \cdot \overline{\psi(\xi)} - \overline{\psi(\xi)}, \end{aligned} \quad (12)$$

Let us examine a special solution of equation (11), which determines the field of displacements due only to the action of force (2). For this reason, we shall assume that  $u_1 = u_2 = 0$  over the perimeter of OM. In this case,  $\varphi(\xi)$  and  $\psi(\xi)$  are found by the following functional equations:

$$\kappa\varphi(\xi) = \frac{1}{2\pi i} \int_{\Gamma} \frac{\omega(\sigma)}{\omega'(\sigma)} \cdot \frac{q'(\sigma)}{\sigma - \xi} d\sigma = V\omega^2(\xi), \quad (13)$$

$$\psi(\xi) = -\frac{1}{2\pi i} \int_{\Gamma} \frac{\overline{\omega(\sigma)}}{\overline{\omega'(\xi)}} \cdot \frac{q'(\sigma)}{\sigma - \xi} d\sigma + \overline{\kappa\varphi(\xi)}, \quad (14)$$

where  $\omega(\xi)$  is conformal transformation reflecting the OM region in plane  $z$  ( $z = x_1 + ix_2$ ) into a single circle in plane  $\xi$ ,

$$\kappa = \frac{3-\nu}{1+\nu}, \quad V = \rho \frac{1-\nu}{2(1+\nu)} (a_1 - ia_2),$$

where  $i$  is an imaginary unit; the line on top signifies complexly conjugate values.

Let us define conformal representation of  $\omega(\xi)$  in the following manner. Since  $\omega(\xi)$  is an analytical function within a circle, let us express it as:

$$\omega(\xi) = \sum_{m=0}^{\infty} C_m \xi^m. \quad (15)$$

On the boundary of the circle  $\xi = \sigma = e^{i\theta}$ , and for this reason the coefficients of expansion (15) can be defined using the following formula:

$$C_m = \frac{1}{2\pi} \int_0^{2\pi} \omega(\theta) e^{-im\theta} d\theta. \quad (16)$$

At given values for  $\omega(\theta)$ , this integral is easy to calculate numerically.

Analysis revealed that it is sufficient to limit oneself to three terms for some OM models in expansion (15):

$$\omega(\xi) = \sum_{m=0}^3 C_m \xi^m. \quad (17)$$

In particular, for the OM described in [6], coefficients  $C_m$  have the following values:

$$C_1 = 3.05 \cdot 10^{-3} \text{ cm}, \quad C_2 = 4.1 \cdot 10^{-3} \text{ cm}, \\ C_3 = -3.3 \cdot 10^{-3} \text{ cm}.$$

Let us express  $\varphi(\xi)$  in the following form:

$$\varphi(\xi) = \sum_{m=0}^6 D_m \xi^m. \quad (18)$$

Substituting (18) in (13), we shall have a system of algebraic equations that define  $D_m$ :

$$D_1 = AV + \bar{B}\bar{V}, \quad D_3 = \frac{1}{\kappa} [(B_1 + b_3 B) V + \\ + b_3 \bar{A} \bar{V}], \\ D_2 = \frac{1}{\kappa^2 - 4|b_3|^2} [\{\kappa(\bar{B}_1 + b_3 \bar{B}) + 2\bar{b}_3 b_3 A\} V + \\ + \{2b_3(\bar{B}_1 + \bar{b}_3 \bar{B}) + \kappa b_3 \bar{A}\} \bar{V}], \\ D_0 = \frac{1}{\kappa} \sum_{n=0}^3 (n+1) \bar{D}_{n+1} b_n, \\ D_m = \frac{V}{\kappa} A_{m-3} \quad (4 \leq m \leq 6), \\ A = \frac{bQ + cZ}{b^2 - |c|^2}, \quad B = \frac{bz + \bar{c}Q}{b^2 - |c|^2}, \\ c = b_1 + \frac{4b_2^2 \bar{b}}{\kappa^2 - 4|b_3|^2}, \\ b = \kappa \frac{2\kappa|b_2|^2}{\kappa^2 - 4|b_3|^2} - \frac{3|b_3|^2}{\kappa}, \\ z = \frac{2\kappa \bar{b}_2 B_1}{\kappa^2 - 4|b_3|^2} + \frac{3\bar{b}_3 B_2}{\kappa}, \\ Q = \frac{4b_2 \bar{b}_3 B_1}{\kappa^2 - 4|b_3|^2}, \quad B_m = \sum_{n=1}^m C_n C_{m-n+1} \\ (1 \leq m \leq 2), \\ A_m = \sum_{n=m}^3 C_n C_{m-n+3} \quad (1 \leq m \leq 3), \quad b_0 = -3 \frac{\bar{c}_3}{c_1} \times \\ \times b_3 - 2 \frac{\bar{c}_2}{c_1} b_1, \\ b_1 = \frac{c_1}{c_1} - 3 \frac{\bar{c}_3}{c_1} b_3 - 2 \frac{\bar{c}_2}{c_1} b_2, \\ b_2 = \frac{c_2}{c_1} b_3, \quad b_3 = \frac{c_3}{c_1}.$$

From equation (14) we have:

$$\psi(\xi) = \frac{1}{\omega(\xi)} \sum_{m=1}^3 Q_m \xi^m, \quad (19)$$

where

$$Q_1 = \kappa [2\bar{D}_0 c_2 + 3c_3 \bar{D}_1] - \sum_{n=1}^3 \bar{c}_n (n+2) D_{n+2},$$

$$Q_4 = 6\bar{c}_1 D_6,$$

$$Q_2 = 3\kappa \bar{D}_0 c_3 - \sum_{n=1}^3 \bar{c}_n (n-3) D_{n+3},$$

$$Q_3 = \sum_{n=1}^2 (n+4) \bar{c}_n D_{n+4}.$$

#### Comparison to Experiment. Discussion

Description of behavior of the OM as an SDP enables us to offer new interpretation to a number of experimental facts and to assess some of the OM parameters.

In general, the obtained solution is rather complicated, and for this reason we shall limit ourselves to consideration of the OM range that meets the condition  $|\xi| \sim 0.1$ . In this case, retaining in (12) only the linear terms for  $\xi$ , we shall obtain after some simple transformations:

$$u_1 = \frac{r}{E} \left[ m_0 \cos \beta + r \right] \frac{m_1^2 \cos^2 \beta + m_2^2 \sin^2 \beta}{\cos(\theta + \gamma_1)}, \quad (20)$$

$$u_2 = \frac{r}{E} \left[ n_0 \sin \beta + r \right] \frac{n_1^2 \cos^2 \beta + n_2^2 \sin^2 \beta}{\sin(\theta + \gamma_2)}, \quad (21)$$

where  $\lg \gamma_1 = \frac{m_2}{m_1} \lg \beta$ ,  $\lg \gamma_2 = \frac{n_2}{n_1} \lg \beta$ ,  $m_j, n_j$   
( $j = 0, 1, 2$ )

for the discussed geometry of OM (see Figure 2) have the following numerical values:

$$\begin{aligned} m_0 &= 2.57 \cdot 10^{-4} \text{ cm}^2, \quad m_1 = 7.26 \cdot 10^{-5} \text{ cm}^2, \\ m_2 &= 2.62 \cdot 10^{-5} \text{ cm}^2, \quad n_0 = 3.34 \cdot 10^{-4} \text{ cm}^2, \\ n_1 &= 1.83 \cdot 10^{-5} \text{ cm}^2, \quad n_2 = 5.89 \cdot 10^{-5} \text{ cm}^2. \end{aligned}$$

In the calculations, it was assumed that Poisson's coefficient equals 0.5.

Young's modulus for OM substance can be evaluated with a given acting force using expressions (20) and (21). Unfortunately, there have not been any experiments to define the displacements of different segments of OM as a function of applied force (for example, gravity), and for this reason, let us proceed as follows. We know [6] that the receptor hair cell functions in a linear mode with about  $10^{-4}$  cm displacements of a hair. It is logical to assume that under natural conditions such displacements are caused by gravity. Assuming in (20) that  $\rho \sim 10^3$  dyne/cm<sup>3</sup>, we shall obtain  $E > 10^3$  dyne/cm<sup>3</sup>. Considering that the mean density of OM is close to density of endolymph, Young's modulus will be smaller; for example, at  $\rho_1 = 0.9 \rho_2$ ,  $E \sim 10^2$  dyne/cm<sup>2</sup> [sic].

The obtained expressions for  $u_1$  and  $u_2$ , with a known Young modulus, enable us to determine the field of displacements of a specific OM region. Knowing the displacement at each point of the OM plane, one can also assess the inclination of receptor cell hairs. Since displacement of a hair in the direction from stereocilia to the kinocilia and in the opposite direction constitutes an effective stimulus for the receptor cell, the projection of displacement in this direction is of interest.

Let us add to our consideration a solitary vector  $\vec{t}$ , which characterizes the direction of morphological polarization of a single cell:

$$\vec{t} = \cos \gamma \vec{i} + \sin \gamma \vec{j}, \quad (22)$$

where  $\gamma$  is the angle between axis  $x_1$  and vector  $\vec{t}$ . Then the projection of displacement in the chosen direction of polarization will appear as follows:

$$U = U_0 \cos(\gamma - \gamma_0), \quad (23)$$

$$\text{tg } \gamma_0 = \frac{u_2}{u_1}, \quad U_0 = \sqrt{u_1^2 + u_2^2}.$$

According to expressions (20) and (21), different parts of the OM will present different displacement and, consequently, different projections in the direction of morphological polarization. If we consider that FSI change  $\Delta f$  is a function of projection of displacement  $\Delta f = f(U)$ , the OM model in question, as an SDP, predicts frequency of impulsation as a function of position of receptor cells on the macula and geometric configuration of the OM. This conclusion is consistent with experimental data [7].

It should be noted that the frequency of neural impulsation was represented in [7] as a function of the projection of force applied to the vector of morphological polarization of an individual cell. However, according to the data in [4], when OM is exposed to a sinusoidal stimulus the impulsation frequency differs from it in phase. If we consider that impulsation is a function of projection of force, no phase difference would be observed, and this is in contradiction to experimental data [4].

One of the distinctions of otolith function is that resting impulsation of various similar neurons is different [7]. Solution (12) does not explain this fact, since with  $a = 0$ ,  $u_1 = u_2 = 0$ . However, this can be explained proceeding expressly from the conception being developed here of an OM as an SDP. Let there be no inertial force acting on the OM-endolymph system. Since the OM



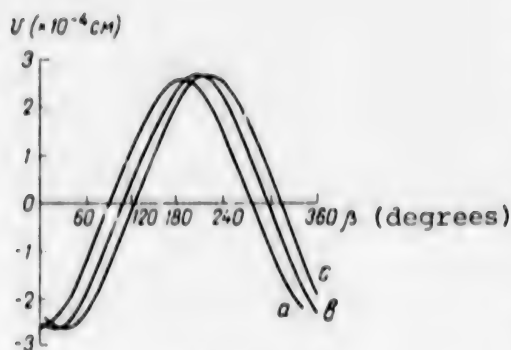


Figure 3.

Projections of displacement  $U$  of various OM points as a function of direction of inertial force

- a)  $r = 0$ ,  $\Theta = \pi/2$ ,  $\gamma = 180^\circ$ ,  
 $U_1 = 0$  cm,  $U_2 = 2.6 \cdot 10^{-4}$  cm
- b)  $r = 0.1$ ,  $\Theta = \pi/2$ ,  $\gamma = 195^\circ$ ,  
 $U_1 = 1.7 \cdot 10^{-5}$  cm,  $U_2 = 2.6 \cdot 10^{-4}$  cm
- c)  $r = 0.2$ ,  $\Theta = \pi/2$ ,  $\gamma = 210^\circ$ ,  
 $U_1 = 3 \cdot 10^{-5}$  cm,  $U_2 = 2.7 \cdot 10^{-4}$  cm

( $U_1$  is projection of displacement when inertial force is perpendicular to vector  $\vec{t}$ ,  $U_2$  is projection of displacement when inertial force is parallel to vector  $\vec{t}$ )

pressure. It should be noted that in this case the submitted solution does not reflect components of displacement as a function of OM configuration and, accordingly, resting FSI. For this reason, this interpretation within the limits of a model of OM as an SDP is merely a qualitative interpretation of the experimental fact under discussion.

According to the morphological map (see Figure 2), the angles of vectors of morphological polarization of cells in the region for which  $|\xi| \sim 0.1$ , are in the range of  $180$  to  $210^\circ$ . This enables us to represent the projection of displacement  $U$  as a function of direction of applied force (Figure 3). From the submitted plots we see that when OM is exposed to a force, the direction of which is perpendicular to the vector of morphological polarization of an individual cell, the projection of displacement and, consequently, impulsation frequency are other than zero. The only exception is case  $a$ , which corresponds to the response in which the projection of displacement  $U$  caused by a force perpendicular to polarization vector  $\vec{t}$  equals zero, which is the result of distributed nature of the system. Using the obtained results, one can demonstrate that, in cases  $b$  and  $c$ , the ratios of  $U_1$  to  $U_2$  equal  $6$  and  $11\%$ , respectively, whereas according to experiment [8] this ratio constituted

is in the endolymph, pressure of which is other than zero, let us determine approximately the stress on the boundary of the OM that arises as a result of action of this pressure in the following manner:

$$-P_0 n_i = \sum_{j=1}^2 \tau_{ij} n_j, \quad (i = 1, 2)$$

where  $n_i$  are components of the normal to OM boundary. The solution of equation (11) under given boundary conditions has the appearance of (3):

$$u_1 = -\frac{1-\nu}{E} P_0 x_1,$$

$$u_2 = -\frac{1-\nu}{E} P_0 x_2,$$

hence, in its base state OM is deformed. Since different parts of OM will have different displacements here, resting impulsation of various similar neurons will also be different in value. Thus, it can be assumed that the observed scatter of impulsation frequency at rest for similar neurons innervating spatially different parts of the macula is the result of the effect of intralabyrinthine

10-15%. Within the limits of the model of OM as a system with concentrated parameters, displacement projection over the direction of morphological polarization differs from zero only under the effect of a force with a projection over this direction.

This question had already been discussed in the literature [8]. Three possible explanations were explored. The first, suggested already in [9], consisted of the fact that nerve fibers innervate at the same time several cells with different morphological polarization which, of course, leads to presence of impulsation other than zero under the effect of a force perpendicular to the vector of morphological polarization of one of the cells. This hypothesis is rejected by quantitative analysis [8]. The second interpretation is that deflection of the hair of an individual receptor cell orthogonally to the vector of polarization would lead to its stimulation. However, in the opinion of the authors of [6], a force perpendicular to the vector of morphological polarization of the cell "elicited virtually no response." Unfortunately, they do not submit the value for the ratio of impulsation frequency when a cell hair is displaced perpendicularly to its polarization vector as compared to parallel direction. For this reason, this interpretation requires further experimental verification. The third explanation is based on the concept of OM in the form of an elastic body. Having obtained experimentally that impulsation frequency is an asymmetrical nonlinear function of direction of compulsive force, the authors assumed that there is a nonlinear relationship between deformation and plate stress. In our opinion, there is an alternative explanation for this fact, which is based on linear theory of elasticity and nonlinearity of the cell's response to deflection of its hair [4, 5]. In reality, a functional OM is, as was shown above, in a deformed state under the effect of intralabyrinthine pressure. The initial displacements thus induced at different points of the OM will be directed toward its center. This displacement will be summated with the displacement evoked by inertial force of corresponding points, or subtracted from it, depending on the direction of this force. In the former case, with initial displacement parallel to the vector of morphological polarization, impulsation frequency will increase and in the latter case, it will decrease. The absolute value of the difference between resting impulsation frequency and forced impulsation frequency will be greater for an individual cell in the former case than in the latter. This interpretation requires special experimental verification, the purpose of which should consist of the following. In the first place, displacements of different OM points as a function of magnitude and direction of applied force must be determined experimentally (analogously to the function obtained when studying the profile of the cupula of the semicircular canals [13]). In the second place, we need a distinct conception about the function of an individual hair cell, in particular about its response to perpendicular and parallel displacement of the hair in relation to the vector of morphological polarization. This would permit us to differentiate between phenomena related to behavior of the OM as a mechanical system and phenomena elicited by the function of receptor cells, and would make it possible to choose the type of OM model that is closest to a real one.

In conclusion, let us note that special solution (12) defining the field of displacement only in response to applied force, which we have submitted here, needs to be supplemented in the future with a solution that describes effects related to interaction between the OM boundary and endolymph.

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## COMBINED EFFECT OF IONIZING RADIATION AND ALTERED GAS ATMOSPHERE ON CENTRAL NERVOUS SYSTEM

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[Article by B. I. Davydov, I. B. Ushakov and V.P. Fedorov]

[Text] When the brain is exposed to ionizing radiation, marked changes may develop rather soon in its structures [20], which lead to neurological disorders [18, 21], as a result of edema, as demonstrated in clinical practice [4] and experimentally [11, 17]. However, the mechanisms of development of these disturbances have still not been sufficiently investigated, while the feasibility of modifying radiation effects in the central nervous system (CNS) by changing the inhaled gas mixture is unjustifiably doomed to failure by some authors [7], and this is in fact in contradiction to the universal law of radiobiology, the oxygen effect, which has been well-studied in other critical systems [16]. At the present time, there is no unequivocal answer to the question of direction of modification of radiation effects in the CNS with use of an altered gas atmosphere. For this reason, we undertook this study on 67 dogs of both sexes weighing 7-12 kg.

## Methods

Before exposure to radiation, vests were placed on the dogs, which were adapted for immobilization of the animals to the walls of a box-stand, in which they were irradiated. The head was immobilized in the anterior compartment of the box by means of two sliding plates with oval slots that firmly encircled the dog's neck. Gamma radiation was delivered to the head or the entire body from a Khizotron unit ( $^{60}\text{Co}$ ) in a dosage of 5 Gy at a dose rate of 4.2 cGy/s along the midline of the body. The coefficient of exposure dose gradient constituted 1.65. When exposing the head, the dose to the shielded part of the body (abdomen) did not exceed 0.5%.

During irradiation, some of the animals were exposed simultaneously to a hypoxic gas mixture (HGM) with 7% (HGM-7) or 10% (HGM-10) oxygen content or to pure normobaric oxygen. Atmospheric air or the gas mixture were pumped through at the rate of  $23.3 \pm 1.18$  l/min. Gas composition in the experimental chamber was monitored in the course of the test by means of gas analyzers (VTI-2 USSR).  $\text{CO}_2$  concentration in the chamber did not exceed 0.3% during the experiment.



Table 1. Changes in total fluid content of different CNS structures of dogs following total-body or local irradiation in a dosage of 5 Gy

Brain structures	Characteristics of experimental animal groups											
	T	H	T	H	T	H	C	T	C	T	C	T
	testing time, min											
	0	0	120	120	300	300	100	300	100	300	100	300
							HGM-7		HGM-10		oxygen	
Spinal cord (cervical)	—	—	—	—	+	+	—	—	—	—	—	—
Medulla oblongata	—	—	—	—	+	+	—	—	—	—	—	—
Pons varolii	—	+	—	—	—	—	—	—	—	—	—	—
Peduncles	—	+	—	—	—	—	—	—	—	—	—	—
Mesencephal. tectum	—	—	—	—	+	+	—	—	—	—	—	—
Cerebellum	—	—	—	—	—	—	—	—	—	—	—	—
Anterior hypothalamus	—	—	—	—	—	—	—	—	—	—	—	—
Posterior "	+	+	+	+	+	+	—	—	—	—	—	—
Thalamus	+	—	+	+	+	+	—	—	—	—	—	—
Caudate nucleus	+	+	+	+	+	+	—	—	—	—	—	—
Hippocampus	—	—	+	—	—	—	—	—	—	—	—	—
Archipallium	+	+	—	+	+	+	—	—	—	—	—	—
Frontal cortex	—	—	—	+	—	—	—	—	—	—	—	—
Sensorimotor cortex	+	+	+	+	—	—	—	—	—	—	—	—
Temporal cortex	—	—	—	—	—	—	—	—	—	—	—	—
Occipital cortex	+	+	+	+	+	+	+	+	+	+	+	+
Parietal cortex	—	—	—	+	+	+	+	+	+	+	+	+
Structures with hypohydration, %	62	56	62	50	34	12	19	50	41	81	77	23
reliability of BHP homeostasis, %												
entire brain	98	98	95	98	43	69	68	76	68	19	95	86
afterbrain	98	99	99	95	37	69	60	97	33	86	87	92
hindbrain	99	100	98	99	59	85	89	90	51	46	90	68
mesencephalon	96	97	100	100	68	43	43	66	98	34	98	87
diencephalon	98	99	100	99	72	97	99	100	76	3	98	93
telencephalon	99	97	90	98	40	65	64	63	77	16	96	86
brain stem	98	98	99	99	43	51	50	81	71	40	91	88
subcortical nuclei	97	94	100	96	81	63	58	81	99	58	99	92
limbic system	98	99	99	100	54	98	99	100	85	1	98	90
cortex	100	100	99	100	30	56	59	44	69	8	94	77
extrapyramidal	98	94	78	95	81	95	83	93	100	100	100	80
thalamo-parietal	100	100	100	100	59	31	38	27	100	100	100	97
thalamo-frontal	100	100	99	100	50	99	98	100	100	0	100	97
caudate-frontal	97	91	69	93	47	94	79	99	100	0	100	97

Key: T) total-body irradiation H) irradiation of head C) control  
 $*p \leq 0.05$ , as compared to the control.

A special series of studies was conducted to determine the possible role of disturbances in the brain's hydration profile (BHP) following total-body and local irradiation of the head. Fluid content was determined by the desiccation method [1]. We examined the following parts of the CNS: spinal (cervical) cord and medulla oblongata, pons, peduncles and tectum of the mesencephalon, cerebellum, anterior and posterior hypothalamus, thalamus, caudate nucleus,



hippocampus, corpus callosum and various parts of the cortex (archipallium, limbic, frontal, sensorimotor, temporal, parietal and occipital). Summary data are listed in Table 1.

An effort was made to assess reliability of BHP homeostasis function in different parts and systems of the brain by means of calculating the general parameter of result of biological experiment [6]. Concurrently, we performed a neurohistological, histochemical, electron microscopic and morphometric studies. Survey sections were stained with hematoxylin-eosin. Neurons and glial cells were demonstrated after Nissl and their processes after Cajal-Favorskiy. Condition of myelin sheaths was assessed according to Weigert-Pal and Marchi. Total protein was demonstrated after Bonhague, glycosaminoglycans after Mowry and RNA after Shi. Succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) were demonstrated by tetrazolium-reductase techniques, while alkaline phosphomonoesterase (APM), acid phosphomonoesterase (AcP) and chymase were demonstrated by azo-combination methods. APM activity was measured by the stereometric method, that of dehydrogenases and protein by two-wave microdensitometry. In the karyometric studies, we measured the large and small diameter of neurocyte nuclei and determined their volume using the formula for an ellipsoid of rotation. We then took logarithms (in cubic micrometers) of nuclear volumes, combined them in groups at class intervals of 0.2 and determined the percentage of each class to plot variation curves. In the course of the study, we counted neurons and glial cells per unit area, percentage of cells with reactive and destructive changes and calculated the neuronal index [15].

For electron microscopy, pieces of brain were fixed by infiltration in 2.5% glutaraldehyde solution with 0.2 M collidine buffer with postfixing in 1% osmic acid solution. After dehydration in ethanol, the material was imbedded in epon-812 epoxy resin. Ultrafine sections were produced on an LKB-III ultratome (Sweden), they were contrasted after [19] and examined under an IEM-100 SX-II electron microscope (Japan).

## Results and Discussion

It should be noted that, on the whole, many reliable changes were observed in cortical elements of the brain. There was gradual increase in hyperhydration of the brain: the number of structures with increased fluid content gradually increased for 5 h. Interestingly, this parameter was higher at all tested times in the case of local irradiation of the head. Accordingly, hypohydration was less marked in brain structures (see Table 1).

The results of morphological studies revealed that reactive, destructive and compensatory-adaptive reactions are demonstrable in the dog brain after both local and total-body irradiation. Maximum polymorphism of changes was referable to the sensorimotor cortex (Table 2). Immediately after uniform total-body irradiation in a dosage of 5 Gy, some morphological changes were observed on the light-optic level, which had a tendency toward building up after 2 h and particularly after 5 h into the recovery period. Following exposure of the head alone the changes were less marked than in the case of total-body radiation. However, already after 2 h, there was an increase in number of cells with reactive and destructive changes. All this led to

significant decline of the neuronal index. Thus, 2 h after irradiation of the head, the changes were more marked in the sensorimotor cortex than after exposure of the whole body to uniform radiation in the same dosage. The findings persisted 5 h into the postradiation period.

Table 2. Characteristics of changes in canine sensorimotor cortex 5 h after exposure to radiation in a dosage of 5 Gy and altered gas atmosphere

Exposure	Neurons				Glia		mast cells, %	APM activity, %	SDH activity, %	LDH activity, %
	per unit area	reactive changes, %	destructive changes, %	neuron index	per unit area	satellite, %				
Biological control	12 ± 0.3	16 ± 0.9	5.6 ± 0.9	2.4 ± 0.1	6.7 ± 0.8	17 ± 1.7	100	100	100	100
Head	11 ± 1.7	22 ± 2.0	9.0 ± 0.5	1.8 ± 0.2	5.3 ± 1.0	11 ± 3.6	93	190	111	—
Total body (TB)	14 ± 0.5	20 ± 1.2	12 ± 1.2	1.6 ± 0.01	2.5 ± 1.4	50 ± 1.4	108	95	75	62
TB + HGM-7	13 ± 0.7	24 ± 0.9	12 ± 1.5	1.5 ± 0.05	9.9 ± 1.6	56 ± 2.5	85	129	78	62
TB + HGM-10	12 ± 2.0	25 ± 1.1	10 ± 1.6	1.5 ± 0.1	5.8 ± 1.0	52 ± 1.8	83	133	—	—
TB + oxygen	11 ± 0.2	16 ± 1.1	14 ± 1.0	1.6 ± 0.1	7.9 ± 0.1	51 ± 3.0	107	91	78	66
HGM-10	12 ± 1.1	20 ± 1.5	9 ± 0.6	1.8 ± 0.1	6.2 ± 0.4	17 ± 4.6	91	111	78	63
HGM-7	11 ± 0.8	25 ± 4.9	16 ± 3.2	1.3 ± 0.2	5.5 ± 1.7	17 ± 2.7	77	126	—	—
Oxygen	13 ± 1.0	14 ± 0.6	10 ± 3.8	2.0 ± 0.1	12 ± 3.1	61 ± 1.3	113	100	81	70

Changes were demonstrable in the cerebellum only 5 h after irradiation. In the case of total-body radiation they were reactive, whereas in the case of exposure of the head there was an increase in number of cells with signs of destruction. Reactive changes were observed in the thalamus and caudate nucleus in the 5th h of the postradiation period only with exposure of the head. In the hippocampus, there was reliable increase in number of cells with both reactive and destructive changes after exposure of the head, whereas with total-body exposure virtually no changes were observed. No significant changes were demonstrable in the medulla oblongata on the level of vagal nuclei with both variants of irradiation.

Thus, on the light-optic level, changes were more marked after exposure of the head to radiation than after total-body uniform exposure. However, in both radiation variants, most neurons and glial cells presented no significant morphological changes on the light-optical level. We also failed to demonstrate significant changes in the vascular system. We did not encounter a single instance of stasis in the microcirculatory system or hemorrhages in brain matter, which confirms the data in [4].

Electron microscopy revealed more profound changes than on the light optic level. They were also more marked after irradiation of the head. Ultrastructural changes were demonstrable the most distinctly 5 h after exposure and they were referable to all elements of the CNS: neurons, interneuronal contacts and structures of the blood-brain barrier (BBB). The most typical findings were reduction in number of neuronal ultrastructures (particularly ribosomes) and swelling of mitochondria, the cytoplasmic reticulum and elements of Golgi's apparatus. Histochemically, there was decrease in activity of redox enzymes and increase in AcP activity. Some cells demonstrated sites of

degeneration in the form of accumulation of lysosomes and residue of organoids. Pyknomorphic neurons with "foamy" cytoplasm were also encountered. After total-body irradiation, such cells were seen more often in the sensorimotor cortex and thalamus, whereas with exposure of the head they were more often found in the sensorimotor cortex and hippocampus. There were particularly noticeable changes in interneuronal contacts. We encountered a rather large number of synapses with degeneration of the clear and focal type [2]. Degeneration of the clear type was encountered equally often in all parts of the CNS with both variants of irradiation, whereas focal degeneration occurred more often in the thalamus. In the latter case, the presynaptic membrane was markedly convoluted and its integrity was impaired.

Several changes were also demonstrable in BBB structures. The microcirculatory vessels were nonuniformly dilated, the endothelium of some capillaries was lighter and contained a moderate number of pinocytotic vesicles. Stable parameters of APM activity are indicative of unchanged transport through the capillary wall. Intercellular contacts were unchanged, although in a number of instances there was increased osmiophilia of contacting parts of plasmolemma. The basal membrane retained its usual ultrastructural organization, but it was more friable in some regions and presented a vague outline. Fine vacuolization was demonstrable in the cytoplasm of pericytes. There was some decrease in number of mast cells as demonstrable with the chymase reaction. Hypertrophied astrocyte islets were consistently encountered. In some places, their membranes were destroyed and edema was formed (perivascular and pericellular).

Table 3.  
Volume of neurocyte nuclei ( $\mu\text{m}^3$ ) 5 h  
5 h after exposing dogs to 5 Gy  
radiation in an altered gas  
atmosphere

Exposure	Sensorimotor cortex (middle layers)	Hippocampus	Cerebellum (Purkinje cells)
Biological control	140 ± 5	221 ± 12	439 ± 30
Head	161 ± 22	235 ± 11	532 ± 42
Total body (TB)	225 ± 17*	156 ± 15	451 ± 65
TB + HGM-10	216 ± 8*	200 ± 10	
TB + HGM-7	161 ± 20	140 ± 9	291 ± 11*
TB + oxygen	118 ± 13	160 ± 12*	151 ± 96
HGM-10	196 ± 11	172 ± 11	
HGM-7	174 ± 12	212 ± 18	235 ± 59
Oxygen	104 ± 10*	278 ± 14	403 ± 24

\*Reliable changes as compared to  
biological control group

Thus, irradiation of dogs (total-body or local) elicits several changes in the CNS 5 h after exposure, the most noticeable of which are disturbances referable to synapse architectonics and BBB elements. However, it should be noted that, with both variants of radiation, the ultrastructural changes were not relevant to CNS function. The vast majority of interneuronal contacts had the usual structure. There was distinct demonstration of microtubules and neurofilaments. Most cellular elements did not have gross ultrastructural changes, and in some cases we were able to also demonstrate signs of a compensation and adaptation: shifting of the nucleolus to the periphery and release of ribonucleoproteins from it, convoluted course of karyolemma and opening of nuclear pores, formation of a cytoplasmic network from the external karyolemma membrane, lysosomal reaction, etc. Activation of the protein-synthesizing system was more marked with total-body radiation, as indicated by the volume of neuronal nuclei, although its changes were in different directions in different parts of the brain (Table 3).

It should be stressed that we never observed "paired neurons," appearance of which some authors [8] believe to be an expression of compensatory and adaptive processes when the brain is exposed to radiation.

Considering the fact that there are very few works dealing with the effects of the gas environment used to modify radiation effects on the CNS [8, 13, 14], we feel it is expedient to dwell on some of our findings.

Pure normobaric oxygen virtually failed to alter BNP 5 h after exposure (95% reliability), whereas HGM led to marked changes (68% reliability for the entire brain), which was equivalent to the results of exposing the head to radiation (see Table 1). There were reliably more structures with hyperhydration with HGM-7. No reliable deviations were demonstrable in any structure with HGM-10.

It is known [5] that inhalation of HGM-10 leads to 1.5-fold drop of oxygen tension in the canine cortex and subcortical region, whereas inhalation of 100% normobaric oxygen is associated with elevation of tension in brain tissue to the same extent.

Thus, 1.5-fold change in oxygen tension in canine brain tissue had little effect by itself on BNP.

Morphological study established that brief hypoxia elicits appearance of cells with reactive and even destructive changes in the canine brain, and the reactive changes are significant in the hippocampus. However, most neurons in all structures of the brain presented no significant morphological changes. Furthermore, 5 h after exposure there was an increase in protein and nucleic acid content of neurocytes. Electron microscopy also failed to reveal gross changes in the CNS. Only a few cells appeared with increased osmiophilia and fine vacuolization of cytoplasm. A typical finding consisted of spiral-shaped membrane complexes formed from the altered cytoplasmic reticulum in the cytoplasm of neurons. Most mitochondria remained unchanged, although one could occasionally encounter swollen and destroyed forms. SDH activity diminished somewhat, whereas LDH activity showed a significant decline in the hippocampus.

We also failed to demonstrate, under hyperoxic conditions, gross changes in specialized elements of neurocytes (synapses, neurofilaments, dendritic spines, etc.). The changes detected in them (swelling of presynaptic terminal, decrease in number of vesicles) are reversible, according to current concepts [2].

HGM-10, as we have already noted, did not elicit significant changes in nerve cells 5 h after irradiation. When oxygen content of the inhaled mixture was reduced to 7%, already on the light optic level more than 40% of the neurocytes of the sensorimotor cortex presented various morphological changes, which led to the most marked decline of neuronal index, as compared to this parameter in experimental groups (see Table 2). Ultrastructural examination of the CNS revealed a number of changes referable to both cellular and vascular elements. They were the most marked in the limbic cortex and hippocampus and least marked in the brain stem. In all these regions, we encountered typical hypoxic neurocytes. They were reduced in size, showed increased osmiophilia of karyoplasm and cytoplasm, their organelles were usually swollen



lending a foamy appearance to the cytoplasm. Pyknomorphic neurocytes were not uncommon (particularly with HGM-7).

Synapses were usually unchanged under the effect of HGM-10, but in some cases there was a decrease in vesicles. Under the effect of HGM-7, there were distinct changes of the light type in interneuronal contacts, and focal degeneration of synapses was also encountered. A disintegrated spine system was not an uncommon finding, and the axonal part of the synapses was overfilled with vesicles.

Several changes were also demonstrable in BBB elements. Thus, there was a decrease in number of juvenile forms of mast cells and swelling of the perivascular astrocyte expansion. Under the effect of HGM-7 one can observe destruction of astrocytes and formation of perivascular and pericellular edema.

Thus, an altered gas atmosphere used to modify effects of radiation on the CNS can in itself elicit a number of changes in the brain. This applies, in particular, to HGM-7, with which the most marked morphological changes in parameters were demonstrated in all experimental groups.

Unlike the separate action of an altered gas atmosphere, which elicits only moderate sequelae in the CNS, the modifying influence of the gas atmosphere on radiation effects, in particular on BHP, was rather distinct. HGM diminished while oxygen augmented the number of structures with hyperhydration by the 5th postradiation hour, which is perhaps indirect evidence of manifestation of the oxygen effect on this parameter. An analogous direction of modification of neurological symptoms was demonstrated in another study [12] with reference to mice following exposure to radiation of the head in doses eliciting changes in the CNS.

A distinct modifying effect of pure mormobaric oxygen was also found upon morphological examination of the brain (see Table 2). First of all, we were impressed by the decrease in number of neurons per unit area of sensorimotor cortex section, which is indicative of late stages of degeneration and rapid lysis of products of cell breakdown. Almost half the Purkinje cells of the cerebellum presented various morphological changes. Synergism of the tested factors was also found upon examination of the hippocampus, caudate nucleus and thalamus.

Ultrastructural studies consistently revealed dark and pyknomorphic cells with a marked astrocyte reaction and neuronophagia. In a number of cells we observed disintegration and breakdown of ultrastructures with formation of myelin figures. Increase in number of lysosomes next to Golgi's apparatus was a typical finding. Contact between neurocytes and glia was encountered rather often. And, in some cases, the glial processes separated neurocytes into fragments, whereas in others there were sites of degeneration in the form of accumulation of lysosomes at the contact points. All this creates favorable conditions for speediest utilization of products of cell breakdown. There was some depression of activity of cell nuclei and redox enzymes.

At the same time, we were able to also find several factors that attenuated the CNS reaction to radiation. Thus, there was less marked swelling of the



perivascular astrocyte expansion and, consequently, less frequent edema. Some improvement was demonstrated in synapse architectonics, particularly in the thalamus and sensorimotor cortex. Presynaptic parts of the synapses appeared less swollen, contained a moderate number of vesicles and little-changed mitochondria. However, here too, we encountered diverse changes in synapses, while degeneration of the clear and focal type was consistently seen in the hippocampus.

The action of a hypoxic gas mixture that modifies radiobiological effects depends on oxygen content. Thus, when animals were exposed to radiation with HGM-10 no changes whatsoever were demonstrable in the CNS, as compared to parameters of animals exposed to radiation under ordinary conditions. We can merely mention some changes in permeability of the capillary wall, as indicated by the elevated APM activity and swelling of the perivascular astrocyte terminal expansion, particularly in the hippocampus. In addition, there were fewer destructive changes in the thalamic synapses, and the changes that were encountered were mostly reactive. However, on the whole, HGM-10 does not have a significant modifying effect on radiation damage to the canine CNS. It should be noted that more optimistic data on this score are cited in [10], but the methods used there are not quite correct and require a cautious approach.

Reducing oxygen content to 7% in the inhaled mixture did not attenuate the effect of radiation on the CNS. Moreover, some increase in number of neurocytes with morphological changes were observed in the sensorimotor cortex and cerebellum. Ultrastructural investigation revealed signs indicative of more profound damage to nerve cells. Thus, pyknomorphic neurocytes subject to breakdown were encountered more often than after radiation alone. Disintegrating mitochondria and a cytoplasmic net, as well as many lysosomes forming sites of local degeneration in some places, were evident in the osmiophilic cytoplasm of such cells. Interestingly, such sites also appeared in the perivascular zone, which was not encountered at this observation time after radiation alone.

Synapse architectonics of different parts of the CNS were virtually the same as with radiation alone, but "adhesion" of vesicles in the center of swollen preterminals was observed somewhat more often. Changes in the postsynaptic region could be demonstrated in some of these synapses, whereas in others it was absolutely unchanged.

The condition of BBB elements was different from their state after radiation alone. There was a decrease in number of young forms of mast cells and more marked swelling of perivascular astrocyte expansions, with the destruction of which there was formation of perivascular and pericellular edema. We observed swelling and disorganization of endothelial ultrastructures, as well as focal depolymerization of glycosaminoglycans in the basal membrane. It is also interesting to note that earlier changes are demonstrable in neurons directly adjacent to capillaries than in neurocytes that make contact with the latter through astrocytes.

The relatively more marked and generally opposite changes, as compared to HGM-7, that occur when animals are exposed to radiation using HGM-10 appear paradoxical at first glance. In all probability, HGM-7 is such a potent

factor (as can be seen from its action alone) that it leads to a certain "breakdown" of adaptation according to biochemical and morphological parameters. In other words, the optimum concentration of oxygen is in the intermediate range of 7-10%.

Table 4. Primary reaction of dogs exposed to radiation in a dosage of 5 Gy and hypoxic gas mixtures

Conditions	Total dogs	Total with vomiting reaction	Incid. of vomit. %	Mean vomiting episodes (arbitr. units)	Mean vomiting start time, min	Mean vomiting end time, min	Mean duration of first reaction, min
Air	15	9	60	3,22±0,81 (75,6)	131,7±17,9 (40,8)	164,4±17,4 (31,5)	32,7±9,7 (89,8)
HGM-7	10	7	70	4,28±0,99 (61,3)	97,9±9,7 (26,2)	151,1±15,2 (26,5)	53,5±14,8 (71,2)
HGM-10	10	8	80	2,25±0,52 (66,1)	130,6±15,9 (34,3)	161,4±10,0 (17,6)	30,8±15,5 (142,8)

Note: Coefficient of parameter variation (%) is given in parentheses.

Of course, it is difficult to make an unequivocal evaluation of the significance of the described changes in the brain when dogs are exposed to radiation in an altered gas atmosphere. As an example, we can cite data pertaining to the primary response of the same animals (Table 4). Hypoxia during irradiation led not only to decrease in frequency of vomiting, but even to some increase. If we combine animals exposed to radiation using HGM-7 and HGM-10, the difference becomes reliable according to the  $\phi$  criterion [3]. This circumstance indicates that use of an altered gas atmosphere to modify the radiation changes in the CNS requires further attentive and in-depth investigation of adverse side-effects.

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## CLINICAL STUDIES

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### EFFECT OF HYPERCAPNIC-HYPOXIC TEST ON CARDIORESPIRATORY PARAMETERS OF INDIVIDUALS WITH NEUROCIRCULATORY DYSTONIA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (manuscript received 22 Jan 87) pp 83-86

[Article by A. S. Nekhayev, V. D. Vlasov, V. K. Stepanov and Yu. I. Andriyenko]

[English abstract from text] Twenty healthy men and twelve patients with hypertensive type neurocirculatory dystonia belonging to the flying personnel were examined. They breathed a hypercapnic-hypoxia mixture formed during rebreathing in a closed circuit without a CO<sub>2</sub> adsorber. In both groups this provocative test produced similar variations of most parameters under study. However in contrast to the healthy men, the hypertensive subjects showed a lower compensatory hyperventilation, a greater increase of blood pressure and cardiac output and a relatively small decrease of total peripheral resistance. Three test subjects displayed sinus arrhythmia. The time of test tolerance in the patients was on the average 20% shorter than in the healthy subjects. The changes can be viewed as an indication that the reserve capability of the cardiorespiratory system declines. The fact that the test is rapid, simple and safe makes it possible to use it during regular medical monitoring of the flying personnel with functional disorders of the cardiovascular system.

[Text] The test involving exposure to moderate hypoxia, which is produced by inhaling a gas mixture with low oxygen content or "ascent" in a hyperbaric chamber, is used for expert evaluation of circulation in flight personnel. This test has not gained wide use in dynamic medical monitoring of individuals with so-called "functional pathology" of the cardiovascular system in view of its methodological and technical difficulty. The rebreathing method, with use of a carbon dioxide adsorber [3] is more suitable for this purpose. The hemodynamic and respiratory effects of hypoxia can be enhanced in this test by hypercapnia [1, 2], which is obtained by eliminating the carbon dioxide adsorber. Hypoxic and hypercapnic chemoreceptor stimuli elicit elevation of arterial pressure, increase circulation volume [cardiac output] and ventilation, as well as blood flow rate and redistribution of blood as a result of



vascular dilatation in organs with intensive metabolism, for example, the heart and brain [11]. It can be assumed that the responses to a hypercapnic-hypoxic atmosphere (HHA) would change, in the presence of functional changes in the cardiovascular system, depending on reactivity of the body and efficiency of control mechanisms. For this reason, the question of the extent to which the test with HHA can be useful in assessing reserve capacities of the circulatory system of individuals with pathology of this system is of both theoretical and practical interest. This was the subject of our investigations.

## Methods

A total of 32 pilots 26-40 years of age participated in the studies, 20 of them were in good health (2d group) and 12 had neurocirculatory dystonia (NCD) of the hypertensive type (1st group). A rubber bag, the initial volume of air in which was produced by means of maximum expiration, i.e., it equaled vital lung capacity (10 l) of the subject, was used for rebreathing. A Fiziolog-M-1 instrument was used to examine external respiratory function and dynamics of heart rate (HR). We recorded parameters of respiration rate (RR), minute respiratory volume (MV), averaged maximum inspiration rate/min (V), effective breathing time/min ( $t$ ). To determine stroke and minute circulation volume (SV and CV), we used tetrapolar rheography calculating the parameters according to Kubicek [12]. Rheograms were recorded in RPB 2-02 and Mingo-graph 82 (Sweden) instruments. The Poiseuille formula was used to calculate total peripheral resistance (TPR). Arterial pressure (BP) was measured after Korotkov. Rhythmocardiograms recorded on a Ritmokardioskop unit were used for analysis of cardiac rhythm. Physiological parameters were recorded before and in the 3d min of rest after the test, and during the test right after the subject's signal warning us that he could not continue breathing (it had been agreed in advance that such a signal would be given 10-15 s ahead of time). The switch to atmospheric air greathing was made after the subject gave a second signal. Upon termination of the test, air was collected from the bag in order to determine the percentage of end carbon dioxide and oxygen content using a Pneumotest (FRG) gas analyzer. In each instance, we recorded the time during which the subject could continue breathing in a closed space. Data were submitted to mathematical processing by the method of Student-Fisher, and Student's  $t$  criterion was used for statistical analysis.

## Results and Discussion

In the baseline study, individuals with NCD showed higher systolic arterial pressure (SBP) and CV, and lower TPR than healthy subjects (see Table). Elevation of resting SBP in patients with NCD is attributed to intensification of adrenosympathetic system activity, increase in plasma catecholamine content, as confirmed by increased 24-h excretion [5]. The absence of hyperventilation in subjects with NCD (MV constituted  $7.8 \pm 0.8$  l/min, versus  $8.5 \pm 0.6$  l/min in healthy subjects) is usually considered a favorable prognostic sign, since hyperventilation may be one of the early signs of development of circulatory insufficiency [9]. During rebreathing, both groups of subjects showed essentially the same dynamics of tested parameters. At the same time, at the end of HHA exposure, those with NCD presented greater increment of SBP and CV ( $33.8 \pm 0.7$  and  $29.5 \pm 0.4\%$ , respectively, versus  $22.7 \pm 0.5$  and  $13.1 \pm 0.3\%$  in the healthy subjects). As we know, under hypocapnic conditions there is



Cardiorespiratory parameters with HHA in healthy subjects and those with NCD (M±m)

Parameter	Group	Baseline data	HHA	Recovery
HR/min	1	78.9±4.2	87.8±5.4	82.4±4.4
	2	75.4±2.7	84.5±3.2	76.2±3.1
SBP, mm Hg	1	142.5±4.3	189.8±6.4	142.3±7.0
	2	131.9±2.9*	161.7±6.2**	131.0±2.6
DBP, mm Hg	1	80.1±4.9	82.6±8.3	83.5±5.2
	2	83.8±2.3	97.0±2.9	83.7±2.7
SV, ml	1	62.6±6.4	64.7±11.5	62.2±8.3
	2	51.8±2.7	50.7±4.5	49.9±4.5
CV, l	1	4.4±0.3	5.7±0.4	5.1±0.7
	2	3.8±0.2	4.3±0.3**	3.8±0.3*
TPR, dyne·s·cm <sup>-5</sup>	1	1815.5±23.1	1639.6±26.2	1613.3±19.8
	2	2060.4±30.6***	2192.1±34.4***	2083.5±32.4***
MV, l	1	7.8±0.8	32.4±3.0	8.9±1.5
	2	8.5±0.6	42.9±2.6**	10.4±1.0
t, s	1	19.8±1.2	28.6±1.9	20.4±0.4
	2	20.1±0.7	28.4±1.0	19.9±1.2
V	1	6.6±0.1	14.1±1.1	8.2±0.5
	2	7.5±0.2***	19.5±1.0***	8.4±0.8
T, s	1	---	289.3±31	---
	2	---	356.6±30	---

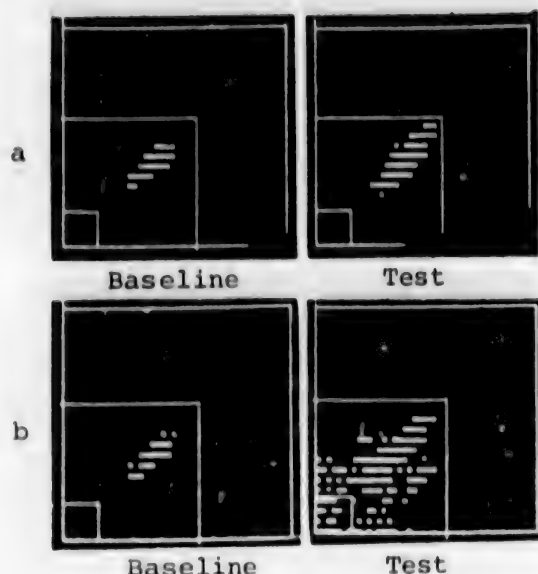
Key: T) respiration time during HHA test. DBP) diastolic blood pressure

\*p<0.05, as compared to parameters of NCD subjects

\*\*p<0.01

\*\*\*p<0.001

increase in catecholamine content [7], which is one of the causes of BP elevation. Considering the pathognomonic significance of higher catecholamine levels for individuals with NCD, it can be assumed that their further elevation with exposure to HHA served as the source of the marked hypertensive reaction. We should have also expected significant compensatory decrease in TPR' however, the decline by about 10% of this parameter, which did occur, was apparently not enough to stabilize BP at a less elevated level. In healthy subjects, TPR increased insignificantly, which can be presumably related to increase in vascular tonus as a result of regional redistribution of blood and decrease in its actively circulating volume. Appearance of sinus arrhythmia merits attention; it was observed during the HHA test in three subjects with NCD, but not at rest before exposure (see Figure). Respiratory acidosis, which occurred due to insufficient compensatory hyperventilation, may be one of the causes of this. Elevation of catecholamine levels is also a provocative factor [6, 8, 10]. In the subjects with NCD, MV showed a mean 4.1-fold increase while averaged maximum inspiratory rate/min showed 2-fold increase (versus 5-fold and 2.6-fold, respectively in healthy subjects), in the presence of virtually the same gas composition of air in both groups at the end of the test (6.5 to 7.2% CO<sub>2</sub>, 10.4 to 12.1% O<sub>2</sub>). Effective breathing time/min increased to about the same extent in the two groups, HR increased by 3-5/min. In our opinion, Maximum possible breathing time in HHA may be of some significance in assessing tolerance to the test. It was about 20% shorter on the average in the NCD cases than in healthy subjects, which is apparently related to increased sensitivity of the respiratory center to hypoxia in the NCD cases [9].



Rhythmocardiogram examples  
 a) in the absence of disease (sinus rhythm)  
 b) sinus arrhythmia

adaptation which, in particular, is confirmed by the results of this study. Use of an examination method including rebreathing is technically simple and safe; at the same time, several questions of the physiological effect of HHA on man require further development and elucidation.

Thus, the cardiorespiratory responses of subjects with NCD and healthy individuals were different under the effect of HHA produced by rebreathing. The elevated BP with inadequate compensation by means of decrease in TPR, appearance of sinus arrhythmia, inadequate hyperventilation and reduction of test tolerance time, which were observed in the individuals with NCD, are signs of inadequacy of adaptive mechanisms. At the same time, it is not deemed possible, on the basis of the findings, to interpret the demonstrated circulatory and respiratory changes as being specific to NCD. In the presence of stress or depletion of regulatory mechanisms, there is usually prevalence of nonspecific symptoms, which are prenosological and characterized by insufficient or satisfactory adaptation to ambient conditions [4]. Under inadequate conditions, there is a greater possibility of detecting signs of de-

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[616.153.1:577.152.1

OXIDATIVE ENZYME ACTIVITY IN HEALTHY SUBJECTS AND INDIVIDUALS WITH  
NEUROCIRCULATORY DYSTONIA DURING GRADED EXERCISE

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21,  
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[Article by Ye. G. Vetrova and I. A. Popova]

[English abstract from source] In response to controlled exercise (50% and 75% of maximum workload) malate dehydrogenase (MDH) and isocitrate dehydrogenase (ICDH) varied similarly in healthy men and in patients with hypertensive and hypotensive type neurocirculatory dystonia. However, in neurocirculatory dystonia subjects MDH increase during exercise and normalization during recovery developed slower than in healthy people. The MDH distribution between mitochondrial and cytoplasmic fractions remained unchanged.

[Text] Inflight functional disturbances of the cardiovascular system may be due to change in hydrostatic pressure, redistribution of blood in organs, muscular atonia, diminished efficiency of the muscle pump, depressed tonus of the sympathetic system, as well as decrease in circulating blood volume [3, 5, 7]. Vegetovascular dysfunction is one of the factors that have an adverse effect on cosmonauts' work capacity. Considering the role of the autonomic nervous system in maintaining homeostasis during use of postural factors, it can be assumed that clinical disorders and biochemical changes, which occur in experiments simulating spaceflight factors, could be reproduced the most distinctly in individuals with neurocirculatory dystonia.

Tissue dehydrogenases, which are involved in conversion of substrates at the early stages of tissular respiration (in the Krebs' cycle), such as NAD-dependent malate dehydrogenase--MDH (EC 1.1.1.37) and NADP-dependent isocitrate dehydrogenase--ICDH (EC 1.1.1.42), are represented in the spectrum of blood enzyme activity. Blood MDH is present in blood in the form of two cytoplasmic fractions (MDH<sub>1</sub> and MDH<sub>2</sub>) and one mitochondrial fraction (MDH<sub>3</sub>).

To increase the objectivity of assessment of the effects on man of spaceflight factors simulated by water immersion and with relative restriction of motor activity, we conducted a study of the above-mentioned enzymes using the opposite stimulus--graded exercise.

## Methods

Dehydrogenase activity was measured in three groups of subjects of about the same age after spending 7 days in the hospital without drug therapy. The 1st group consisted of 10 healthy men, the 2d, of 10 people with neurocirculatory dystonia of the hypertensive type and the 3d, 9 people with neurocirculatory dystonia of the hypotensive type. They exercised on a cycle ergometer in recumbent position at 50 and 75% of maximum load, as determined by pulse rate with consideration of age, weight and sex. Venous blood was drawn with a catheter 2 days before the study, just before exercise, at the time of reaching 50 and 75% of maximum load and 30 min after working on the ergometer. MDH and ICDH activity was determined by the enzyme spectrophotometric method using the test kits of the Beringer firm (FRG); distribution of MDH isozymes was determined by electrophoresis in polyacrylamide gel. The obtained data were submitted to statistical processing by means of determining reliability of quantitative differences between results obtained at different times on the same group [1].

## Results and Discussion

The following results were obtained in the baseline period from two examinations: total MDH and ICDH activity in the three groups of subjects was in the range of the conventional physiological norm. Examination of relative amounts of MDH isozymes in individuals with neurocirculatory dystonia of the hypertensive type (2d group) revealed reliable ( $p < 0.02$ ) increase in cytoplasmic fraction MDH to a mean of 35%, versus the normal 15-30% (Table 2). According to the literature [2], patients with grade IIA essential hypertension (persistent hypertension without functional impairment of organs) showed reliable increase in cytoplasmic fraction MDH<sub>1</sub> content with unchanged level of mitochondrial isozyme MDH<sub>3</sub>. With grade IIB and III hypertension (with involvement of internal organs), there was increase in concentration of mitochondrial isozyme MDH<sub>3</sub> due to decrease in cytoplasmic fractions MDH<sub>1</sub> and MDH<sub>2</sub>. This was associated with disturbances in tissue metabolism, change in enzyme spectrum of tissues, redistribution of activity between cytoplasmic and mitochondrial isoforms of MDH. Evidently, cytoplasmic MDH fractions appear in blood at the early stages of disturbances in cellular structures and functions. An increase in amount of mitochondrial MDH fraction, however, is indicative of more profound destructive changes in tissues, which are observed in the presence of tissular hypoxia at times of crisis in patients with essential hypertension and in the presence of uncompensated essential hypertension, when increase in overall MDH activity and significant increase in mitochondrial MDH fraction are observed. In this study, we examined individuals with neurocirculatory dystonia of the hypertensive type, rather than people with persistent essential hypertension; at this stage of the disease no marked metabolic disturbances in tissues are observed, and this was reflected only by an increase in amount of cytoplasmic MDH<sub>1</sub> fraction in blood serum.

In individuals with neurocirculatory dystonia of the hypotensive type (3d group), there was a tendency toward decrease in cytoplasmic MDH<sub>2</sub> fraction to 18% (versus the norm of 20-40%) with concurrent increase in concentration of mitochondrial fraction MDH<sub>3</sub>. Due to the small number of tests made, it is impossible to draw a definitive conclusion as to the distribution of MDH isoforms in the presence of neurocirculatory dystonia of the hypotensive type;



however, the demonstrated changes in distribution of mitochondrial and cytoplasmic MDH isozymes could be typical of the individuals in this group.

Table 1. Blood serum MDH and ICDH activity

Enzyme	Group	Baseline	Before exercise	50% load	p	75% load	p	30 min after exercise	t <sub>1</sub>	F <sub>2</sub>
MDH	1	3.4 ± 0.8	2.5 ± 0.5	3.8 ± 0.6	< 0.01	5.0 ± 1.3	< 0.001	4.2 ± 1.1	< 0.02	< 0.001
	2	3.1 ± 0.7	3.4 ± 0.6	3.5 ± 0.8	< 0.01	5.6 ± 0.9	< 0.01	5.1 ± 1.0	< 0.01	< 0.01
	3	2.1 ± 0.3	2.2 ± 0.4	2.9 ± 0.4	< 0.01	3.2 ± 0.7	< 0.02	2.1 ± 0.1	< 0.01	< 0.01
ICDH	1	47 ± 4.2	46 ± 3.2	67 ± 8.9	< 0.02	56 ± 4.1	< 0.001	16 ± 1.6	< 0.01	< 0.05
	2	67 ± 9.0	53 ± 4.6	74 ± 7.9	< 0.05	78 ± 9.1	< 0.01	65 ± 6.1	< 0.02	< 0.05
	3	70 ± 6.9	60 ± 7.1	74 ± 8.6	< 0.001	80 ± 9.3	< 0.01	70 ± 7.6	< 0.02	< 0.05

Key: p) reliability of differences between data obtained during exercise and parameters before exercise

p<sub>1</sub>) probability of differences between data obtained during exercise as compared to parameters obtained 30 min after exercise

p<sub>2</sub>) same for data obtained before exercise, as compared to parameters demonstrated 30 min after exercise

Note: The norm for MDH is 48-96 IU/ml and for ICDH 0.7 IU/ml

Table 2. Distribution of blood serum MDH isozymes (%)

Group	Isozyme	Baseline	Before exercise	50% load	75% load	30 min after exercise
1	MDH <sub>1</sub>	23.3 ± 1.2	22.3 ± 1.1	20.5 ± 2.2	20.1 ± 1.2	19.4 ± 1.5
	MDH <sub>2</sub>	29.1 ± 0.7	28.9 ± 0.6	28.5 ± 0.9	28.1 ± 1.1	28.0 ± 1.8
	MDH <sub>3</sub>	47.6 ± 1.9	48.8 ± 1.3	51.0 ± 2.1	51.8 ± 1.2	52.6 ± 2.9
2	MDH <sub>1</sub>	34.1 ± 3.1	34.0 ± 3.9	33.8 ± 2.9	35.0 ± 3.9	38.1 ± 4.1
		p < 0.02				
	MDH <sub>2</sub>	27.8 ± 1.6	30.2 ± 2.3	30.5 ± 2.6	31.0 ± 2.8	27.9 ± 1.3
3	MDH <sub>3</sub>	38.1 ± 3.5	35.8 ± 4.3	35.7 ± 4.5	34.0 ± 4.8	34.0 ± 5.2
	MDH <sub>1</sub>	20.0 ± 1.4	19.5 ± 1.7	20.0 ± 1.3	19.5 ± 1.5	18.6 ± 1.1
	MDH <sub>2</sub>	18.1 ± 2.3	16.5 ± 1.6	18.3 ± 2.1	18.2 ± 2.4	18.5 ± 2.1
	MDH <sub>3</sub>	61.9 ± 2.7	64.0 ± 2.4	61.7 ± 2.3	62.3 ± 1.9	62.9 ± 2.5

Note: Norm for MDH<sub>1</sub> is 15-30%, MDH<sub>2</sub>--20-40% and MDH<sub>3</sub>--40-60%.

Table 1 lists the changes in enzyme activity during exercise. At the time 50% of maximum load is reached MDH activity increased significantly and reliably in all three groups, and continued to increase, reaching a maximum at 75% of maximum load in the 2d and 3d groups of subjects. As compared to baseline activity, the increase constituted 47 and 34%. In the control group, peak activity was observed upon reaching 50% of maximum load (65% increase). A decrease in MDH activity was observed in all subjects 30 min after exercise, the level reaching the baseline in the healthy men of the 1st group and remaining elevated in subjects with neurocirculatory dystonia.

Determination of relative amounts of MDH isozymes failed to reveal reliable changes in distribution of activity among mitochondrial and cytoplasmic fractions (Table 2).

The dynamics of ICDH activity during graded exercise resembled the changes in MDH activity (see Table 1). A reliable successive increase was demonstrated in ICDH activity of subjects of the 1st, 2d and 3d groups in response to 50 and 75% of maximum load, constituting 100, 65 and 45%, respectively, of the baseline. By the 30th min after exercise, there was significant decline of ICDH activity in all 3 groups of subjects. Baseline levels of activity were recorded only in individuals with neurocirculatory dystonia of the hypotensive type.

Consequently, a heavy exercise load elicits appreciable elevation of levels of Krebs' cycle dehydrogenases in blood serum of both individuals with neurocirculatory dystonia and healthy subjects. However, MDH activity 30 min after exercise on the cycle ergometer did not revert to the baseline in individuals with neurocirculatory dystonia of either the hypotensive or hypertensive type, and we observed some lag in reaching maximum MDH activity levels, as compared to the control group.

Numerous data have been published to the effect that there is an increase in activity of serum enzymes, such as MDH [5], creatine phosphokinase, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase [4, 8, 9] during various forms of exercise. The phenomenon of increase in blood enzyme activity can be explained as follows: during exercise the body needs more energy, which causes increase in oxygen and glucose uptake; in intensively functioning muscles there is activation of metabolic processes aimed at meeting the increased energy requirement, which probably leads to increase in permeability of histohematic barriers and release of enzymes into intercellular fluid and blood over the gradient of their concentrations. As shown here, selective permeability of cell membranes to mitochondrial and cytoplasmic MDH isozymes does not change, as indicated by the stable distribution of MDH in mitochondrial and cytoplasmic fractions.

It follows from our findings that, in addition to the above mentioned enzymes, there is increase in blood ICDH activity with graded exercise, an enzyme that limits the rate of conversion of substrates in Krebs cycle. This could be due not only to change in membrane permeability and release of the enzyme into blood, but its activation by the ADP excess, which is formed when there is intensified hydrolysis of ATP in a functioning muscle.

Thus, intensive exercise (to 75% of maximum), which stimulates processes of energy metabolism, leads to an increase in activity of blood serum oxidative dehydrogenases.

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## BRIEF REPORTS

UDC: 612.13-06:612.76].014.46:615.217.24

### HEMODYNAMIC EFFECTS OF BETA-ADRENERGIC BLOCKING WITH OBSIDAN IN CLINOSTATIC AND ORTHOSTATIC POSITIONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (manuscript received 5 Sep 86) pp 90-94

[Article by G. S. Belkaniya, M. V. Galustyan, V. A. Dartsmeliya and A. N. Demin]

[Text] The typological distinctions of circulation in healthy individuals and patients with arterial hypertension in orthostatic position were established in previous studies [4, 5]. It was shown that the differentiated hemodynamic types reflect phasic regulation of central and peripheral circulation when changing from normotensive to hypertensive modes of controlling arterial pressure (BP). In subsequent studies [3], data were obtained that confirmed the thesis that the regulatory distinctions of the gravity factor of circulation in clinostatic and orthostatic position modify appreciably the hemodynamic changes that occur under psychoemotional stress, as well as under the effect of physical and pharmacological factors. Attention was called to the fact that differences in reactivity of the cardiovascular system to the most diverse factors may be due to the types of circulatory regulation in orthostatic position [4].

Determination of reactivity of the cardiovascular system as a function of position of the body and state of regulation of gravity factor of circulation in orthostatic position is particularly important to cardiological practice, if we consider that use of highly active cardiovascular agents is made under both resting clinostatic conditions (bedrest, intake of agents at night) and in the active period of the day, when the patient, when unrestricted, has an orthograde body position under different conditions (he is seated, standing or walking) with daytime intake of agents. Determination of this function is also important to space medicine practice, since we know that in weightlessness, in the presence of which cosmonauts are mentally and physically active and exposed to diverse factors including intake of cardiovascular drugs when medically indicated, there are specific and appreciable changes in gravity conditions.

Our objective here was to determine the relevance of distinctions of circulatory regulation in clinostatic and orthostatic positions to hemodynamic effects of the  $\beta$ -adrenergic blocker, obsidan.

## Methods

This study was conducted with the participation of 34 volunteer subjects. The subjects were given one therapeutic dose of obsidan (40-80 mg) in the presence of stabilized circulation in orthostatic position (hemodynamic parameters were recorded 15-20 min after preparing patients in initial standing position) and clinostatic position (15-20 min after patients assumed recumbent position). Hemodynamic changes in the 15th, 30th, 40th and 60th min after intake of the drug were assessed as percentage of baseline clinostatics-obsidan and orthostatics-obsidan.

Standardized characteristics of the main parameters of central hemodynamics in clinostatic and orthostatic positions were obtained on 147 healthy men. The type of circulatory regulation in orthostatic position was established according to change in circulation volume [cardiac output] (CV) as related to resting clinostatic position [4, 5]. The type was diagnosed as hypokinetic (or I) when CV in orthostatic position was less than 94% (108 men; 74%), eukinetic (type II) when it was 94-106% (18 people; 12%) and hyperkinetic (type III) at values exceeding 106% (21 people, 14%) (Figure 1).

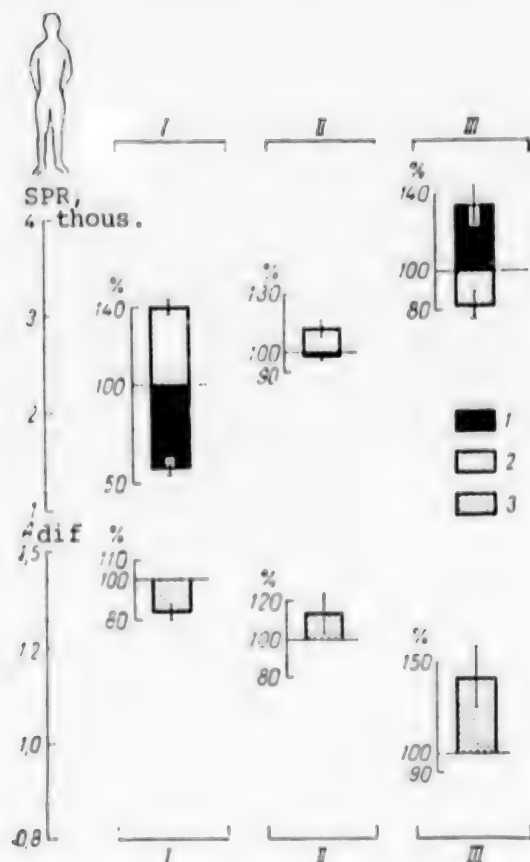


Figure 1.

Standardized characteristics of the three types of central hemodynamics in orthostatic position

The profiles of correlations of CV and TPR with the 3 (I-III) types of circulation in orthostatic position are arranged in accordance with absolute SPR (dyne·s·cm⁻⁵) in clinostatic position; typological correlations of contractile function are shown in accordance with absolute  $A_{dif}$  ( $\Omega \cdot s \cdot cm^{-1}$ ) on thoracic rheogram in clinostatic position

BP was measured by the indirect Korotkov method. Tetrapolar rheography was used to determine the following principal parameters of central and peripheral hemodynamics: stroke volume of the heart (SV), CV, total (TPR) and specific (SPR)



peripheral vascular resistance, mean arterial pressure ( $BP_m$ ). Contractile function of the myocardium (CF) was established according to amplitude of differential thoracic rheogram ( $\Delta d_{if}$ ), which reflects cardiac output rate. Arterial influx to extremities ( $AI_e$ ) and viscera ( $AI_v$ ) was determined according to amplitude of rheograms of the abdomen, thighs and legs; venous influx of blood to the heart (VI) was determined accordance to difference between changes (percentage) in peripheral (abdomen, limbs) and central impedance ( $Z_p - Z_c$ ). Peripheral venous efflux (VE) was calculated from the algebraic sum of relative changes in arterial influx ( $\Delta AI$ ) and basal impedance ( $\Delta Z$ ) of examined peripheral regions (abdomen, extremities). Peripheral resistance to arterial influx (RAI) was estimated from the difference between relative changes in AI and SV. The obtained data were processed by methods of variation and nonparametric statistics.

## Results and Discussion

Figure 1 illustrates the main hemodynamic correlations with the three distinguished types of circulation in healthy subjects in orthostatic position. As we see, type I in orthostatic position is characterized by decline of CV and increase in TPR, type III by increase in CV and decline of TPR and type II holds an intermediate position between hypokinetic and hyperkinetic according to changes in CV and TPR. It is important to note that all three types are reproduced under normotensive conditions (healthy subjects), with arterial hypertension of grade I and II [5]. This shows that there is a basic similarity of operating mechanisms of circulatory regulation in orthostatic position in the presence of these states. At the same time, baseline parameters of hemodynamics in resting clinostatic position differ appreciably: marked decrease in CV with progressive increase in TPR is reflected in the step-by-step change both among types of circulation in orthostatic position and successively at higher SPR levels from normotensive regulation of BP to hypertensive with grades I and II hypertension. CV in resting clinostatic position decreases progressively [5].

The latter circumstance presents substantial difficulties in demonstrating reactivity of the cardiovascular system as a function of hemodynamic parameters in clinostatic position, i.e., the position in which circulation is traditionally examined in humans. On the other hand, it is virtually impossible to match a homogeneous sample of patients with respect to hemodynamics, according to clinostatic parameters. Some difficulties may also arise when testing healthy individuals, due to the marked age-related pattern of changes in basic parameters of central hemodynamics, for example, CV, TPR and others [2, 8, 9]. Evidently, this is the principal cause of contradiction of data in the literature concerning dependence of reactivity of the cardiovascular system on initial type of hemodynamics, usually determined in resting clinostatic position. This brief typological description of circulation in clinostatic and orthostatic positions is necessary to validate a differential approach to evaluation of reactivity of the cardiovascular system. The latter is based on orientation toward types of circulation in orthostatic position, which enables us to overcome the above-mentioned difficulties. The data we obtained on the hemodynamic effects of obsidan serve as additional confirmation of this.

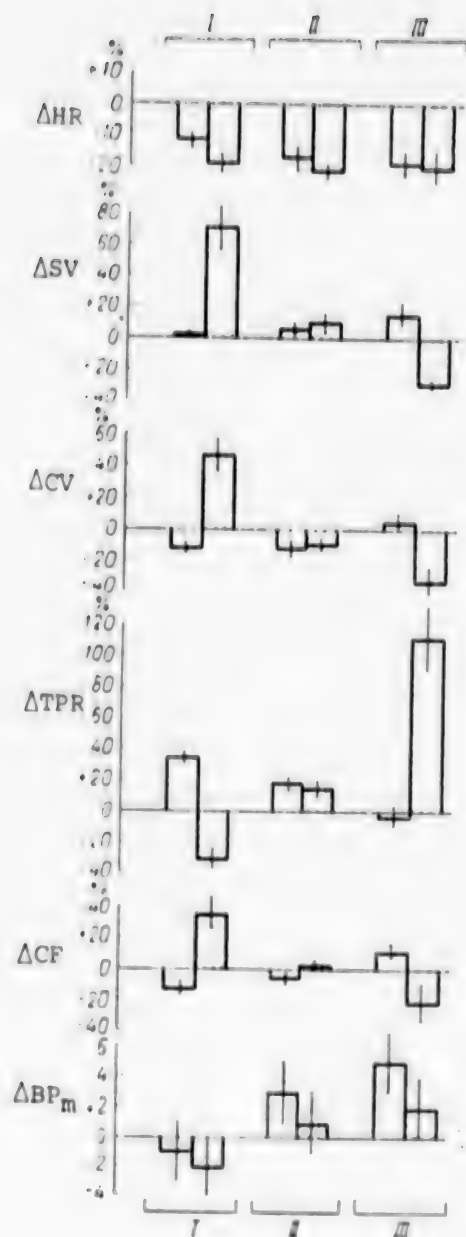


Figure 2.

Changes in main parameters of central hemodynamics with  $\beta$ -adrenergic blocking with intake of obsidan in recumbent and erect positions as related to the three types of circulation in orthostatic position I-III) types of hemodynamics  
White bars--recumbent position, hatched--erect

the findings was performed comparing the hemodynamic effects of  $\beta$ -adrenergic blocking to types of orthostatic circulation.

Table 1.

Correlations between baseline SV and CV in clinostatic and orthostatic positions and changes in presence of  $\beta$ -adrenergic blocking with intake of obsidan in recumbent and erect positions

Parameter	Conditions		Coefficient of correlation
	basel.	obsidan	
SV	R	R	-0.7*
	R	E	0.0
	E	E	-0.5**
	E	R	-0.9*
	R	E	-0.6*
CV	E	E	0.2
	E	R	0.6*
	E	E	-0.9*

\*  $p < 0.001$

\*\*  $p < 0.02$

Key: E) erect  
R) recumbent

The sample examined (34 people) was distributed as follows according to type of circulation in orthostatic position: type I--21 people (62%), type II--6 (18%), type III--7 (20%). Correlation analysis of the entire sample, with respect to baseline circulatory parameters and hemodynamic effects of  $\beta$ -adrenergic blocking (Table 1), revealed a high negative linear correlation between SV and CV in clinostatic position and changes in these parameters with intake of obsidan in recumbent position. However, there was no correlation between SV and CV in clinostatic position, on the one hand, and hemodynamic response to obsidan, on the other, when it was taken in erect position. At the same time, there was a reliable and high correlation between changes in SV and CV in orthostatic position, i.e., type of circulation in orthostatic position, on the one hand, and the hemodynamic effects of obsidan when taken both in erect and recumbent position, on the other hand. This is indicative of the deciding significance of state of circulation and its regulation in orthostatic position to reactivity of the cardiovascular system, in particular to obsidan. For this reason, further analysis of

According to the data illustrated in Figure 2, the hemodynamic effects of  $\beta$ -adrenergic blocking are distinctly in the opposite directions with intake of obsidan in recumbent and erect positions with extreme types of circulation in orthostatic position, according to SV, CV and TPR. With type I circulation, there was decrease in CV in clinostatic position and increase in orthostatic position, whereas with type III, the opposite was demonstrated, i.e., hemodynamic reactions to intake of obsidan in recumbent position corresponded to CV and TPR changes in orthostatic position, and were opposite in direction to those observed with intake of the drug in erect position. Thus, according to one of the most important parameters of central hemodynamics--cardiac output--we demonstrated, in the first place, that the hemodynamic reaction with the body in different positions when taking obsidan is a distinct function of the circulatory type in orthostatic position and, in the second place, this reaction is in opposite directions in clinostatic and orthostatic positions with the same type of hemodynamics.

How is such a differentiated pharmacodynamic effect of beta blocking with obsidan on circulation in clinostatic and orthostatic position implemented? Slowing of heart rate is a common result of taking obsidan. This effect was observed in both resting clinostatic position (13, 17 and 18% decrease in HR with types I, II and III circulation, respectively) and orthostatic position (19, 21 and 19% decrease, respectively). The main consequence of slower HR was improvement of cardiodynamics, as manifested by increase in SV in both positions (recumbent and erect) with all three types of orthostatic circulation. The only exception was circulation type III, with which there was maximum increase in SV in clinostatic position (by 16%), whereas in orthostatic position SV dropped by 26% (see Figure 2). Improvement of venous hemodynamics

Table 2.

Changes (%) in parameters of peripheral circulation in presence of  $\beta$ -adrenergic block with intake of obsidan in recumbent (A) and erect (B) positions as related to different types of circulation in orthostatic position

Parameter	Orthostatic circulat. type					
	I		II		III	
	A	B	A	B	A	B
VI <sub>e</sub>	12	15*	16	15	18*	18*
VI <sub>v</sub>	11	17	12	14	12	14
VE <sub>e</sub>	16	19	18	21	21	19*
VE <sub>v</sub>	15	17*	12	11	2	11
AI <sub>e</sub>	11	10	13	16	18	18**
AI <sub>v</sub>	12	0	12	11	11	8
RAI <sub>e</sub>	13	16*	-25	17*	11	-11**
RAI <sub>v</sub>	11	176	-5	17***	25	-21**

+) reliable differences between parameters for types I and III

\*p<0.01, between parameters with subjects recumbent and erect

\*\*p<0.02      \*\*\*p<0.05

(Table 2) was the peripheral reflection of optimization of the hemodynamic systole-diastole profile. In all instances of increased SV, there was improvement of both VE from the extremities (VE<sub>e</sub>--by 16-37%) and VI<sub>e</sub> of blood in ongoing control of hemodynamics of the chronotropic component of cardiac function. It should be stressed that the demonstrated direction of hemodynamic effects of  $\beta$ -adrenergic blocking is quite consistent with the typological distinctions of the heart's inotropic function in baseline states in clinostatic and orthostatic positions (see Figure 1). Intensification of CF in the presence of  $\beta$ -adrenergic blocking with intake of obsidan increased from type I circulation to types II and III (see Figure 2), concurrently with progressive decline of baseline SF level (according to A<sub>diff</sub>) with these types in clinostatic position (see Figure 1), whereas the reverse was

observed in orthostatic position. Consistently with the most marked decline of  $A_{diff}$  on the thoracic rheogram with type I circulation in orthostatic position,  $\beta$ -adrenergic blocking with intake of obsidan in erect position was associated with increase in CF, SV and CV (see Figure 2). With type III circulation, intake of obsidan in erect position led to decrease in CF, and consequently in SV and CV (see Figure 2).

It was previously reported [4, 5] that, with type I circulation, relative hypodynamia of the heart is associated with marked increase in TPR, whereas with type III there is increase in CF, SV and CV, and decrease in TPR. These findings are distinctly shown in Figure 1. Evidently, it is expressly for this reason that  $\beta$ -adrenergic blocking in clinostatic position, i.e., under baseline conditions of maximum mobilization of inotropic function, lowers CV, whereas in orthostatic position, in the presence of regulatory decrease in vascular capacity, optimization of cardiodynamics is associated with marked increase in SV and CV. The deciding importance of the vascular component of regulation of circulation with type I circulation in orthostatic position is clearly manifested by increase in RAI by 64% in the presence of a  $\beta$ -adrenergic block, in spite of the increase in SV and CV. As we know, aside from active vascular reactions, one observes the usually hemodynamic correlations: increase in cardiac output is associated with increase in peripheral arterial blood flow. And, although  $AI_e$  did increase (by 10%) in orthostatic position with  $\beta$ -adrenergic blocking, this increase was not consistent with the extent of increase in cardiac output according to SV (70%), and for this reason RAI also rose (by 64-76%). Thus,  $\beta$ -adrenergic blocking demonstrates quite graphically the deciding significance of increase in vascular tonus to regulation of circulation with type I in orthostatic position.

With type III circulation and initially high TPR (SPR) in clinostatic position, optimization of cardiodynamics by intake of obsidan in recumbent position was also associated with increase in SV and CV. However, in orthostatic position, when the cardiac component of regulation is the main one for this type,  $\beta$ -adrenergic blocking, which "clips" the main mechanism of compensation for the orthostatic circulatory factor, lowers SV and CV. The changes in parameters of peripheral circulation were the opposite of those seen with type I (see Table 2). It should be stressed that the changes in parameters of peripheral circulation were always more prominent and marked in the lower extremities (legs and thighs) than in the abdomen with all types of hemodynamics. This is indicative of more active participation (particularly in orthostatic position) of the vascular system of the limbs in peripheral regulation of circulation. As for type II circulation in orthostatic position, here the hemodynamic changes after intake of obsidan were of an intermediate nature, as compared to types I and III, and were consistent with the substance of this type--most stabilized as well according to hemodynamic changes in orthostatic position, as compared to resting clinostatic position (see Figure 1).

Accordingly, the hemodynamic effects of obsidan in recumbent and erect positions were mild and did not differ appreciably from one another according to virtually all parameters of central and peripheral hemodynamics, with the exception of heart rate (see Figure 2).



As for such a surely controlled parameter as BP, it should be noted that, even with mild changes in it when recumbent and erect, they were in the same directions for the different types. Moreover, with type I,  $BP_m$  dropped in both clinostatic and orthostatic positions, whereas with types II and III it rose. The differences between types I and III circulation in orthostatic position were particularly graphic and reliable (see Figure 2). Elevation of  $BP_m$  with use of the  $\beta$ -adrenergic blocker with expressly type III circulation in orthostatic position stresses to some extent its significance as the end phasic state in either mode of BP regulation and additionally validates the previously formulated conception that type II and, particularly, type III circulation in orthostatic position are transient states toward regulation of the cardiovascular system in more hypertensive modes [5].

Analysis of hemodynamic effects of  $\beta$ -adrenergic blocking with intake of obsidan in recumbent and erect positions offers additional evidence of the fact that the distinguished circulatory types in orthostatic position are not some static states, but that they are based on appreciably different mechanisms of regulation of central and peripheral circulation. The latter was manifested rather clearly in the demonstrated typological distinctions of reactivity of the cardiovascular system to  $\beta$ -adrenergic blocking. Distinct and basically different hemodynamic effects of the latter when obsidan is taken in recumbent and erect positions stress the appreciable differences in regulation of circulation and reactivity of the cardiovascular system in clinostatic and orthostatic positions. The demonstrated typological dependence of hemodynamic effects of  $\beta$ -adrenergic blocking in clinostatic and orthostatic positions makes it possible to predict expected hemodynamic changes and suggests differentiated use of drugs of this class. The importance of our findings is particularly manifest in therapeutic correction of BP, SV, CV and TPR, and shows us that it is necessary to consider the type of circulation in orthostatic position and conditions under which the product is taken (clinostatic or orthostatic). Consideration of these factors will permit prediction of the probable pharmacological activity of  $\beta$ -adrenergic blockers and to prescribe an agent in a differentiated manner, depending on conditions of intake, the main one being the circadian rhythm (mode) of the patient's position.

The demonstrated dependence of hemodynamic effects on body position and typological distinctions of circulation in orthostatic position does not apply exclusively to obsidan; rather, it also extends to other factors and pharmacological agents [3]. Analysis of hemodynamic effects of obsidan is justified by the extensive use of  $\beta$ -adrenergic blockers in clinical practice, in the treatment of essential hypertension, cardiac insufficiency, ischemic heart disease and a number of other conditions [7].

At the present time, a conception is being formed to the effect that body position has an effect on the pharmacokinetics of various agents [9]. The result of our studies enables us to expand this conception, i.e., transfer it to pharmacodynamics which may be, as shown in this article for obsidan, in the opposite direction with regard to the end effect, depending on position of the body in which the pharmacodynamic activity of some agent or other is effected. This conception is particularly important to chronotherapy and chronopharmacology, since it discloses concretely the significance of body position as one of the most significant biorhythmological conditions of man's vital functions related to regulation of circulation according to the gravity factor.



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## BOOK REVIEWS

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### NEW BOOK ON METABOLIC ASPECTS OF SPACEFLIGHT STRESS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 21, No 6, Nov-Dec 87 (signed to press 23 Oct 87) p 95

[Review by N. N. Demin of book "Metabolicheskiye aspekty problemy stressa v kosmicheskom polete" [Metabolic Aspects of Stress During Spaceflights] by R. A. Tigranyan, Moscow, Nauka, 1985, 224 pages]

[Text] Although there is a vast literature already dealing with stress, in-depth investigation of expressly biochemical mechanisms of processes that develop under stress is only beginning. And we should stress in particular that there has been very little study of metabolic changes under stress in the nervous system, which is directly involved in both its triggering mechanisms and organization of its entire pattern, including adaptive phenomena. As for metabolic aspects of the problem of stress under spaceflight conditions and, first of all, such an unusual state for all living organisms on our planet as weightlessness, this theoretically and practical issue is discussed in this monograph for the first time in the worldwide literature.

A comprehensive survey of the results of 12 years of investigations is offered in this fundamental work by the prominent biochemical specialist, Professor R. A. Tigranyan; these studies were pursued (on rats) both by the author and his colleagues, and the Czech researchers, R. Kvetnansky and L. Macho, who worked with them; the staff of the Laboratory of Functional Neurochemistry at the Institute of Physiology imeni I. P. Pavlov, USSR Academy of Sciences, also participated in the neurochemical studies of all inflight experiments.

The results of a study of the effects of such states as hypokinesia, repeated immobilization, rotation in a drum and emotional stress under ground-based conditions served as the basis for the author's comparative analysis of experimental data. The information obtained is presented in the first two chapters. Paying special attention to biochemical parameters of activity of the adreno-sympathetic system and opioid peptide and polyamine levels in the central nervous system, the researchers also studied some parameters of lipid metabolism, DNA and RNA content, thiol groups, activity of cholinesterases and several enzymes of bioenergetic metabolism. The facts they established are of great interest. In particular, we should like to call attention to data, which are perhaps of more general relevance rather than solely practical implications, to the effect that phenazepam [tranquilizer of benzodiazepine

class] premedication can largely eliminate some of the neurochemical disturbances elicited by immobilization; phenazepam also eliminates neurochemical changes associated with paradoxical sleep phase deprivation.

All these new data definitely constitute a substantial contribution to the study of biochemical and endocrinological correlates of stress.

The principal material of the monograph (chapters 3-7), however, consists of detailed presentation of the results of studies of biochemical parameters in the presence of stress-related changes in experimental animals flown in space for 18.5-22 days in 1973-1979 aboard 5 artificial earth satellites of the Cosmos series. In one of these biosatellites, the animals were exposed in flight to ionizing radiation; in another, a group of rats was exposed to artificial earth gravity (by means of constant rotation in centrifuge). Some of the animals flown in a biosatellite (Cosmos-1129) were submitted to immobilization stress for 150 min/day for the first 6 postflight days. The results of similar studies of intact animals (vivarium control) and animals kept on the ground but under conditions that were entirely identical to those of "flight" rats (synchronous control) served as a control for the data characterizing the flight animals. The data obtained from the synchronous control studies made it possible to single out, to some extent, expressly the effects of weightlessness as such in the flight group.

There is a comprehensive "Conclusion" at the end of the monograph, in which the author offers a summary critical discussion of all of the vast experimental material presented in the preceding chapters. In general, in spite of the fragmentary nature of this material, due to many objective difficulties, and contradictory nature of some findings that make it difficult to interpret them unequivocally, the author validated his opinion that animals present with biochemical signs of a combination of acute stress following spaceflights, due to the conditions of landing, and elements of mild chronic stress during the actual spaceflights. The rather prolonged, for rats, period of weightlessness, hypokinesia and emotionally dramatically impoverished environment are apparently the stress factors associated with flying on biosatellites. As we have already indicated, the effects of the last two factors could have been taken into consideration in studies of the "synchronous control" rats.

On the whole, the monograph by Professor R. A. Tigranyan contains numerous utterly unique data, which he was able to obtain by unique experiments under weightless conditions aboard biosatellites. This information was instrumental in the development of space biology. Herein lies the great value of the monograph.

The monograph is illustrated rather fully: there are illustrations of equipment used, numerous graphs with concrete experimental data. A lengthy bibliography is appended to the monograph.

This book is intended for biochemists, physiologists and pathophysiologists concerned with the problem of stress, as well as specialists working in the field of space biology and medicine.

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